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CERTAIN ENVIRONAL AND NUTRITIONAL FACTORS AFFECTING APHANOMYCES ROOT ROT OF GARDEN PEA[1]

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INTRODUCTION

The *Aphanomyces* root rot (*Aphanomyces euteiches* Drechs.) has been an important disease of pea (*Pisum sativum* L.) in the canning crop in Wisconsin and numerous other States for the past 20 years or more. Earlier studies by Jones and associates (*9, 10*)[2] and by members of the New Jersey Agricultural Experiment Station (*6*; *13, Rpt. 52*) emphasized the importance of soil moisture as well as temperature in their influence upon the development of the disease. More recently Walker and Musbach (*20*) found little or no infection in the greenhouse in soil from a severely diseased field when the moisture level was kept low while at higher moisture contents the disease was very severe.

Efforts to find a practicable control for this disease have been only partially successful. Investigators at the New Jersey Station in a series of reports (*5, 11, 12, 13, 14*) have shown that heavy applications of complete fertilizers are effective in reducing the severity of infection. They also found that the nitrogen compounds used in the fertilizer mixtures were much more influential in reducing root rot than the phosphorus and potassium components. No benefit resulted when the fertilizer was applied after infection had taken place. Walker (*19*) in Wisconsin and Geach (*3*) in Australia have obtained similar decreased infection with the use of complete fertilizers. Walker and Musbach (*20*) showed that applications of fertilizer containing nitrogen reduced root rot damage in the field somewhat more than corresponding applications without nitrogen. They also found that greater protection was effected when the fertilizer was applied in the drill at the time of planting than when it was applied in a furrow at one side of but removed a slight distance from the seed.

The purpose of the present investigation was to examine more closely the relation of certain environmental factors to the development of the disease, and to study the effect of mineral nutrition on its incidence and severity.

MATERIALS AND METHODS

In the study of the effect of nutrition on the disease a constant-flow sand-culture method was used. The nutrient solutions were allowed to drip at the rate of 600 to 800 cc. per day into 8-inch flowerpots filled

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[2] Italic numbers in parentheses refer to Literature Cited, p. 19.

with washed, sterilized silica sand. The inside surface of the pot was varnished to prevent diffusion of salts. Each pot was equipped with an intermittent siphon which allowed the nutrient solution to rise within 2 inches of the surface before draining. The time required for each pot to fill and drain varied from 24 to 36 hours.

The nutrient solution used throughout was a modification of that described by Hoagland and Snyder (7). In addition to the "normal" solution, solutions containing extra quantities of one of the elements N, P, and K and solutions lacking one of these elements were used. The ratio of each element in the "excess" solutions to that in the normal solution was approximately: N, 1.15 to 1; P, 2.0 to 1; and K, 1.28 to 1. A trace of each of the essential minor elements was added to all solutions. The reaction of all solutions was maintained between pH 5.5 and 6.2. The composition of the various nutrient solutions used is given in table 1. In the experiments involving higher concentrations of the nutrients, NaCl was added so that the proportions of the major elements could be varied without changing the total salt concentration.

TABLE 1.—*Chemical composition of the nutrient solutions at the basic concentration used in the sand-culture experiments*

Chemical	Cubic centimeters of molar solutions used to make up 10 liters of nutrient						
	Normal	+K	−K	+P	−P	+N	−N
$Ca(NO_3)_2$	[1] 50.0	50.0	50.0	50.0	50.0	50.0	
$CaCl_2$							50.0
KNO_3	50.0	50.0		50.0	50.0	50.0	
KCl		16.7			10.0		50.0
$NaNO_3$			50.0			22.2	
NaCl [2]	22.2	5.5	22.2	12.2	22.2		22.2
$MgSO_4$	20.0	20.0	20.0	20.0	20.0	20.0	20.0
KH_2PO_4	10.0	10.0		10.0		10.0	10.0
NaH_2PO_4			10.0	10.0			

[1] 50 cc. of a M/1 salt solution in 10 liters equals 0.005 M concentration.
[2] Not used in the dilute nutrient-solution experiments.

Inoculum was obtained by growing the organism in pea decoction in 250 cc. Erlenmeyer flasks. About 25 wrinkled-type pea seeds were placed in each flask, 100 cc. of water was added, and the flasks were plugged and autoclaved for 2 hours. Each flask was inoculated with a fragment of an agar culture of the fungus, and the organism was allowed to grow for 10 to 12 days. The zoospore suspension was obtained by removing the mycelial mats, washing them first in running tap water for 2 hours, and rinsing them in distilled water. They were then placed in shallow pans with just enough water to cover them. Abundant zoospore formation followed within 6 to 8 hours.

Inoculation was made by adding 100 cc. of the zoospore suspension to each pot. In the first experiments the siphon drains were plugged at the time of inoculation and the sand was flooded with the nutrient solution. After 24 hours the plugs were removed and the nutrient was allowed to flow through in the normal manner. In the experiments in which the higher salt concentrations were included, small 1 mm. bore siphons were placed over the edges of the pots after the sand was saturated and the nutrient was allowed to continue dripping into the

pots. With the ends of the small siphons placed under the sand, the siphon column was not broken and the nutrient solution continued to flow into the pots without causing them to flood. The pots were drained after 24 hours.

The Wisconsin Perfection variety of pea was used throughout all experiments, except where indicated. Eighteen seeds were planted in each pot.

DISEASE SYMPTOMS AND METHOD OF DISEASE RATING

In sand cultures the first symptoms of the disease appear on the roots within 4 or 5 days after inoculation. Water soaking spreads above and below the initial infection zone without appreciable discoloration in the area of infection. The water-soaked tissue is firm at first, but it gradually becomes yellowish and soft, darkening with age and eventually collapsing and disintegrating. About 4 days after the underground symptoms may be found, water soaking appears in the stem above the sand level and progresses an inch or more up the aerial portion of the stem.

The extent of disease development was recorded when all or nearly all plants in the dilutest solution in the respective series showed severe above-ground symptoms. Plants were removed from the sand and divided into five arbitrary classes based on the severity of disease and designated as follows: O, No disease; 1, slight water soaking on epicotyl or on primary or secondary roots; 2, moderate water soaking of primary root or epicotyl, with or without slight darkening of the infected tissue, which remains firm; 3, infected areas extensive, darkened, and becoming soft, but without collapse of tissue; 4, extensive water soaking and discoloration with collapse and disintegration of part or all of the infected tissue; plants dying previous to inspection were rated in this class.

A disease index calculated on the basis of the above classes appeared to be a more accurate indication of the extent of infection than the percentage of infected plants, since it afforded a means of indicating the relative severity in combination with the percentage of plants affected. The index was calculated by using the class figures as weighted values. To obtain the index the number of individuals in each class was multiplied by the class number, the sum of the products of each class multiplied by 100, and this figure divided by 4 times the total number of plants. Thus, when all the plants were healthy, the rating was 0, and when all plants showed severe infection, the rating was 100, while intermediate grades were represented by intermediate index figures.

ENVIRONMENTAL STUDIES

TEMPERATURE IN RELATION TO GROWTH OF THE FUNGUS

Plates of potato-dextrose agar adjusted to a neutral reaction were inoculated in the center with 5-mm. disks from the periphery of an actively growing 4-day-old Petri-dish culture of *Aphanomyces euteiches*. Four replicates were placed at each of eight temperatures ranging in 4° intervals from 8° to 36° C., inclusive. Increments of radial growth were measured daily.

The results are shown graphically in figure 1. The greatest daily increase and total colony growth occurred at 28°. No growth occurred at either extreme temperature. From the minimum to the optimum there was a steady increase in rate of growth with increase in temperature. Above 28° the growth rate dropped rapidly. The straight-line trends in the graph indicate that growth was not retarded by the accumulation of staling or other metabolic products of the fungus.

REACTION OF MEDIUM IN RELATION TO GROWTH OF THE FUNGUS

The reaction series was set up by using various quantities of H_3PO_4, and of mono-, di-, and tri-sodium phosphate solutions, each with 0.1

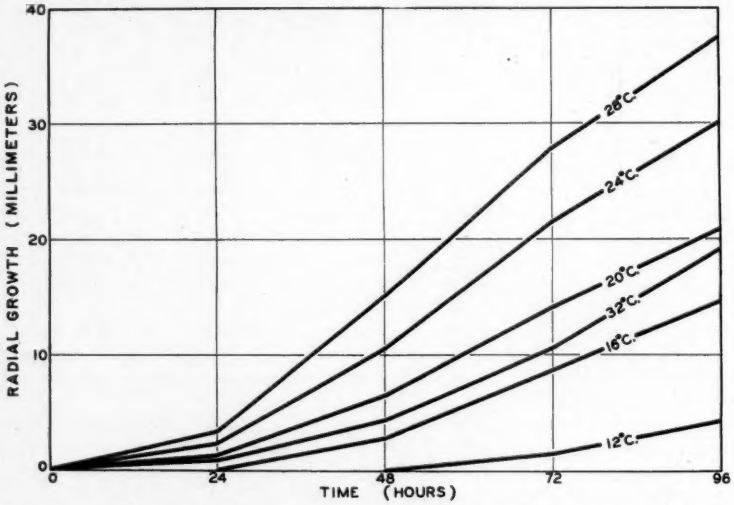

FIGURE 1.—The effect of temperature on growth of *Aphanomyces euteiches* on potato-dextrose agar.

cc. of concentrated H_2SO_4 added, according to the method described by Tilford (*16*). The quantity of phosphates for each pH level was 20 cc.; the amount of each fraction was varied to produce the desired pH level. Each 20-cc. phosphate mixture and a 200-cc. quantity of potato-dextrose agar (1.7 percent agar) was sterilized separately and thoroughly mixed just before pouring the plates. One plate of each pH level was melted, diluted with three parts of distilled water, and the pH determined with a glass electrode. The plates were inoculated in the same manner as in the temperature series. Increments of growth were measured daily and the curve of growth plotted after 4 days.

The results are shown graphically in figure 2. The fungus exhibited a tolerance to a wide range of acidity, with the limits at about pH 3.4 and slightly above pH 8.0. A broad optimum range from about pH 4.5 to 6.5 occurred, with an apparent isoelectric point at pH 5.9. The daily increment of growth at each pH value remained quite con-

stant, indicating no apparent drift in the reaction of the medium or any interference of staling or other metabolic products of the fungus.

EFFECT OF SOIL TEMPERATURE ON INFECTION

The soil-temperature range at which root rot infection can occur has been found by Jones and Drechsler (*9*) to lie between 10° and 30° C., with an optimum somewhere between 15° and 30°. Further study of this important environmental factor was undertaken to determine more exactly the rate of disease development at different soil temperatures. Twenty-four soil-temperature cans were filled to within 1½ inches of the top with clean, sterile silica sand, and each can was equipped with a siphon for drainage purposes. Fifteen seeds were

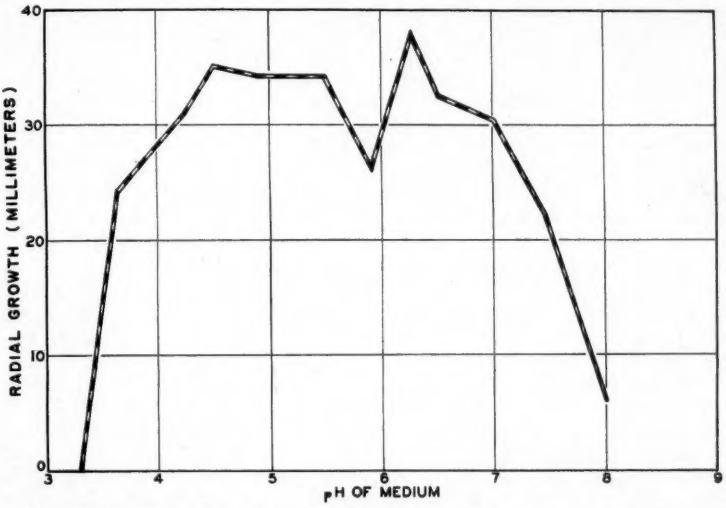

FIGURE 2.—The effect of initial pH of potato-dextrose agar on growth of *Aphanomyces euteiches*.

planted in each can and watered with a balanced nutrient solution of one-tenth the salt concentration described in table 1. All cans were placed at a temperature of approximately 24° in order to secure uniform plant growth. Every 3 days these were drained and fresh nutrient was added. Two days before inoculation four cans were placed in each of six soil-temperature tanks in which the temperatures were maintained at approximately 12°, 16°, 20°, 24°, 28° and 32°, so that the sand in each case did not vary more than 1°. The air temperature varied between 20° to 24°.

Fourteen days after planting, each can was saturated with nutrient and 100 cc. of a zoospore suspension were added. The cans were then partially drained in order to draw the inoculum around the roots, but sufficient moisture was left at the surface for infection to take place. Notes were taken in the manner described for the nutrient studies.

In the 24° and 28° tanks infection was visible 4 days after inoculation. The experiment was terminated 11 days after inoculation when nearly all plants at these two temperatures were infected. The results (table 2) show a definite optimum for disease development at 24° and 28°. No infection was found on plants at 12°, while at 16° some infection did occur. The rate of disease development at the various sand temperatures appeared to parallel quite closely the rate of growth of the organism on agar.

TABLE 2.—*The effect of sand temperature on Aphanomyces root rot development as shown by the extent of disease development 11 days after inoculation*

Disease index at the temperature (°C.) indicated					
12°	16°	20°	24°	28°	32°
0	12.5	51.6	90.4	86.4	55.6

EFFECT OF SOIL MOISTURE ON INFECTION

Others (*5, 9, 10, 20*) have noted that root rot infection is most severe when the soil moisture is high. Observations in Wisconsin during the past three years (1938, 1939, 1940) have indicated that the disease has been more severe in the seasons of abundant rainfall.

For the purpose of obtaining more information on the effect of soil moisture on root rot, an experiment employing two moisture levels was made. The soil was taken from a naturally infected field near Clyman, Wis. It was mixed thoroughly and two portions were adjusted to 45 and 75 percent of the water-holding capacity, respectively. The saturation point of the soil was reached when the moisture content was 43 percent of its dry weight. To maintain a fairly constant moisture content, Wisconsin soil-temperature cans watered every third day were used. A 2-inch layer of ground cork was placed above the soil to reduce evaporation from the surface. Six replicates were included at each moisture level and the arrangement of the cans was randomized. The cans were kept at 20° to 25° C. after 15 seeds had been planted in each. The plants were dug for inspection 30 days later.

TABLE 3.—*The effect of high and low soil moisture on Aphanomyces root rot infection as shown by the extent of disease development 30 days after planting*

[Cans kept at 20° to 25° C. after 15 seeds had been planted in each]

Soil moisture in percentage of water-holding capacity	Percentage of plants infected	Disease index
45	[1] 1.4	0.7
75	[2] 72.0	52.7

[1] 70 plants in 6 replicates.
[2] 82 plants in 6 replicates.

The results are shown in table 3. A statistical analysis of the results was not made because of the extreme difference in the amount of infection between the two series. It is evident from both the disease index and the percentage of infected plants that the higher moisture marked-

ly favored disease development. At 45-percent water-holding capacity, there was a very small amount of infection, indicating that this moisture content of the soil is approximately the minimum at which infection can take place in this soil. This is somewhat higher than the results of Haenseler (*5*), who found that the minimum for infection occurred at about 30 percent of the water-holding capacity of the soil he used, when inoculated by means of a zoospore suspension. Although not determined experimentally, a somewhat higher moisture level than 45 percent of the water-holding capacity could undoubtedly have been maintained in soil used in the present investigation without severe infection.

NUTRITIONAL STUDIES

Although the beneficial action of fertilizers in reducing root rot has been demonstrated in infected soil, the nature of this action has not been explained. Carpenter (*2*) and LeBeau (*8*) showed that high nitrogen increased the root rot of sugarcane caused by *Pythium* spp., and that adequate phosphorus decreased it. Vanterpool (*17*, *18*) demonstrated that the browning root rot of wheat occurred in phosphorus-deficient soil and that applications of phosphorus reduced its severity. Broadfoot and Tyner (*1*), studying the *Helminthosporium* root rot of wheat under controlled conditions, observed that any treatment which resulted in the production of undernourished plants favored disease development. The work of these investigators indicates that unbalanced nutrition predisposes sugarcane and wheat to the causal organisms concerned. Workers at the New Jersey Experiment Station (*11*, *Rpt. 57*) in field studies over a period of several years found that increase in yield of peas on root-rot-infested soil was almost directly proportional to the amount of fertilizer applied even when the rate was as high as 2,400 pounds per acre. This was interpreted as indicating that the control of root rot was a contributing factor to higher yield in addition to the direct effect of nutrients on plant growth, especially at the high fertilizer levels. Because a more adequate explanation of the relation of host nutrition to root rot development in peas was desirable, further investigation of this question was undertaken.

RELATION OF SOIL FERTILITY TO DISEASE DEVELOPMENT

In order to obtain a further check on the effect of nitrogenous fertilizers, a field test was made in 1940. A 1-acre area was chosen in the field just mentioned in which root rot had caused a complete crop failure the previous year. Each plot was 24 by 100 feet in dimension; each treatment was made on three replicates arranged in randomized order. The treatments consisted of an unfertilized check, 2–12–6 fertilizer at the rates of 333, 534, 760, and 1,108 pounds per acre, respectively, and 0–12–6 at the rate of 1,094 pounds per acre. The 2–12–6 fertilizer was a commercial preparation, and the 0–12–6 mixture was compounded from Tennessee Valley Authority superphosphate and commercial 50-percent K_2O. The fertilizers were applied broadcast on recently prepared soil and harrowed in promptly about 2 weeks before planting. Profusion variety was planted on May 8 by means of a seed drill. Throughout the season rains were frequent and the soil was thus kept at a high moisture content, a condition which favored root rot development.

On June 12 random samples, each containing from 40 to 50 plants, were dug from each plot and rated on the same scale as was used in the sand-nutrient series. Twelve days later, on June 24, a second sample, and on July 9 a third sample of plants was dug and rated. The results of the first two inspections were analyzed statistically and are given in table 4.

TABLE 4.—*Effect of fertilizer treatments on naturally infested soil in the field on the development of Aphanomyces root rot on Profusion peas sown on May 8*

Fertilizer treatment	Rate of application in pounds per acre	Mean disease index—	
		35 days after planting	47 days after planting
None		63.70	97.50
2–12–6	333	43.50	97.50
2–12–6	534	45.53	94.53
2–12–6	760	28.50	89.93
2–12–6	1,108	21.20	81.30
0–12–6	1,094	46.57	92.80
Minimum significant difference (5-percent point)		20.77	5.82

The results at 35 days show clearly that disease development in the unfertilized check was significantly greater than that which occurred when 2–12–6 fertilizer was applied at the rates of 760 and 1,108 pounds per acre. At the lower rates of 2–12–6 and at the 1,094-pound application of 0–12–6 there was a little less disease than in the unfertilized plots, but the difference was not significant. A comparison of the 0–12–6 fertilizer application with the corresponding 2–12–6 treatment shows a significant difference in favor of the nitrogen-bearing fertilizer. At 47 days the root rot had increased markedly and the decrease in disease at the 760- and 1,108-pound treatments with 2–12–6 was barely perceptible. At the time of the third field inspection all plots were completely and severely infected, although even at that time the plants in the plots receiving 1,108 pounds of 2–12–6 fertilizer were visibly taller and greener than those in the other plots. The crop was so poor in all plots, however, that yields were not taken. Although from the standpoint of commercial control the results were disappointing, the trend was nevertheless in general agreement with those previously observed (*3*, *5*, *11*, *12*, *13*, *14*, *19*, *20*).

DILUTE NUTRIENT-SOLUTION EXPERIMENTS

In the first series of sand-culture experiments the previously described solutions were applied at one-tenth the basic concentration. In addition to the solutions lacking N, P, and K, a solution lacking S was also employed. Although the nutrient solutions used were quite dilute, it was hoped that the degree of variation in the balance between the major elements would show whether the incidence of root rot was influenced by the nutrition of the host plant.

Four pots were used for each nutrient. Two weeks after planting all were inoculated with a zoospore suspension of *Aphanomyces euteiches* of an undetermined spore concentration. At the end of the first experiment the series was repeated. Inoculation this time was made 3 weeks after planting. In both experiments, above-ground

symptoms appeared within 7 days and all plants were dead within 14 days after inoculation. Regardless of the nutrient employed, infection was equally rapid and severe.

Peas were again planted in the pots used in the second experiment without sterilizing and washing the sand. In this case the inoculum was largely in the form of oospores in the numerous fine roots left in the sand when the previous crop of infected peas was removed, rather than the introduced zoospores which brought about the initial infection. The seedlings emerged normally, but infection was visible 5 days after emergence. All plants were dead within 12 days. Again no effect of nutrient variation was discernible.

On the assumption that the form of nitrogen might have an influence on the disease, one experiment was set up to determine the effect of NH_4 ions as compared with NO_3 ions. The NO_3 solutions was the same as the normal solution used in the experiments described above. In the NH_4 solution the $Ca(NO_3)_2$ and KNO_3 were replaced by $CaCl_2$ and KCl, respectively, and an equal number of nitrogen atoms was added in the form of NH_4Cl. The pots were inoculated 2 weeks after planting. Disease development and severity were equal in both nitrate and ammonium solutions, indicating that at the nutrient concentrations used, neither form of nitrogen had any effect on disease development.

From the foregoing experiments, it was evident that with the nutrient concentration employed, the development and severity of the disease were independent of the nutrient-ion balance, or of the two types of nitrogen ions.

RELATION OF INCREASED NUTRIENT CONCENTRATION AND ION BALANCE TO ROOT ROT INFECTION

With the failure of the low-concentration experiments to show any influence on root rot, it became obvious that the fertilizer action was dependent upon factors other than nutrient-ion balance alone. Since the previous experiments had been made at a very low concentration of nutrients, it seemed advisable to determine whether differences resulting from varied nutrient-ion balance might not occur at higher concentrations. In order first to observe the effect of increased nutrient concentrations, only the complete nutrient solution was used.

In the previous experiments the basic solutions (table 1) were diluted to one-tenth concentration. The concentrations hereafter are referred to as multiples of the basic solution. Thus, the dilute solution mentioned above is designated as 0.1H, the basic as 1H, double concentration as 2H, etc. The arrangement of the sand cultures was the same as in the previous experiments. The inoculation technique used has already been described, and in the following experiments the concentration of the inoculum was standardized at 7,500 to 8,000 zoospores per centimeter of inoculum. One pot in each group of four was left uninoculated.

In the first experiment normal nutrient solution at concentrations of 0.1H, 1H, 2H, and 3H were used. Thus, the highest concentration was 30 times as strong as the nutrient used in the previous sand-culture experiments. As soon as infection appeared it became evident that at the highest nutrient concentration there was a distinct decrease in amount of disease as compared with that in the 0.1H nutrient concentration (fig. 3). The plants were dug for inspection when all the

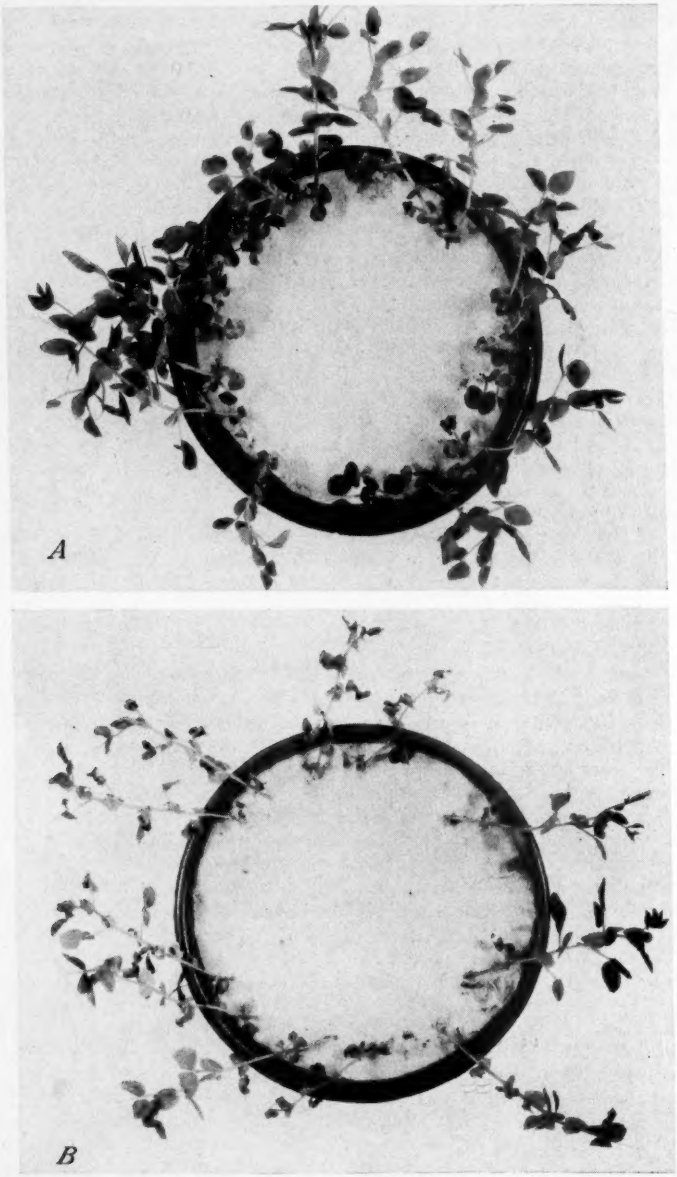

FIGURE 3.—Pea plants 18 days after inoculation with *Aphanomyces euteiches: A*, Plants grown in a high concentration of nutrient solution (3H); *B*, plants grown in a low concentration of nutrient solution (0.1H). The plants in *B* are all severely diseased and most have already died; of those in *A* only one showed any above-ground signs of disease at the time of the photograph.

plants in the 0.1H nutrient showed above-ground disease symptoms. The results (fig. 4, experiment 1) show a striking decrease in the amount and severity of root rot infection at the higher nutrient concentrations. The decrease in the severity appeared in direct proportion to the concentration of the nutrient.

In order to verify the findings in the above experiment, another series was run. Again the same relation between the severity of disease and the concentration of nutrients resulted (fig. 4, experiment 2). Since the 3H solution did not completely control the disease, however, still another experiment was made in which the concentrations 0.1H, 1H, 2H, 3H, 4H, and 5H were used. The same

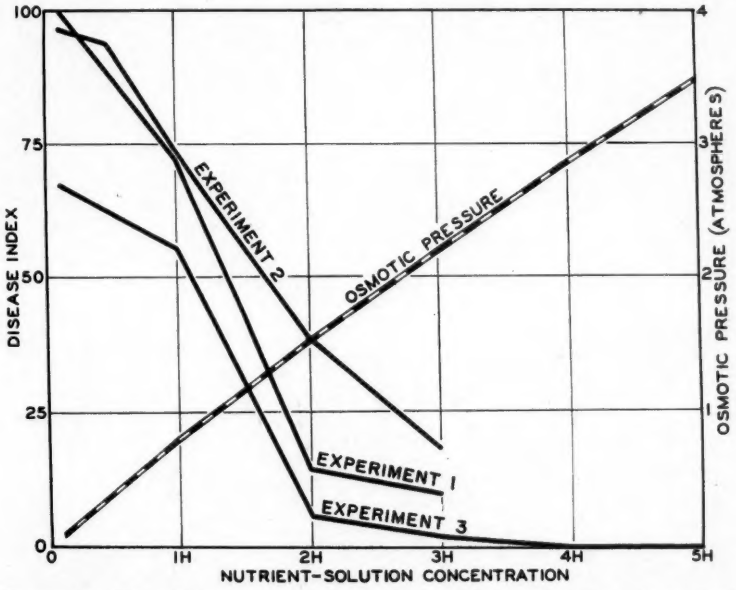

FIGURE 4.—The relation of salt concentration to osmotic pressure of the nutrient and to the development of *Aphanomyces* root rot of pea. Representative plants from experiment 3 are shown in figure 5. Explanation of nutrient-solution concentration symbols will be found in the text.

relative decrease in disease with increased concentration occurred. At the two higher concentrations, 4H and 5H, no infection whatsoever could be found (fig. 4, experiment 3; fig. 5). In this experiment the infection was not so severe in any of the nutrients, although the experiment was allowed to run about 10 days longer than the first two. For this reason the possibility remains that a small amount of infection might take place at the 4H and 5H concentrations. However, from the results of this experiment and the general relation found between concentration and infection in all three experiments, it is highly probable that the zero point for infection, under the conditions of these experiments, would be found at a concentration between 3H and 5H.

The marked reduction in the amount and severity of root rot with increase in concentration of the solution again raised the question of the relation of the nutrient elements to the disease. Although the relative proportions of all elements in the normal solution were the

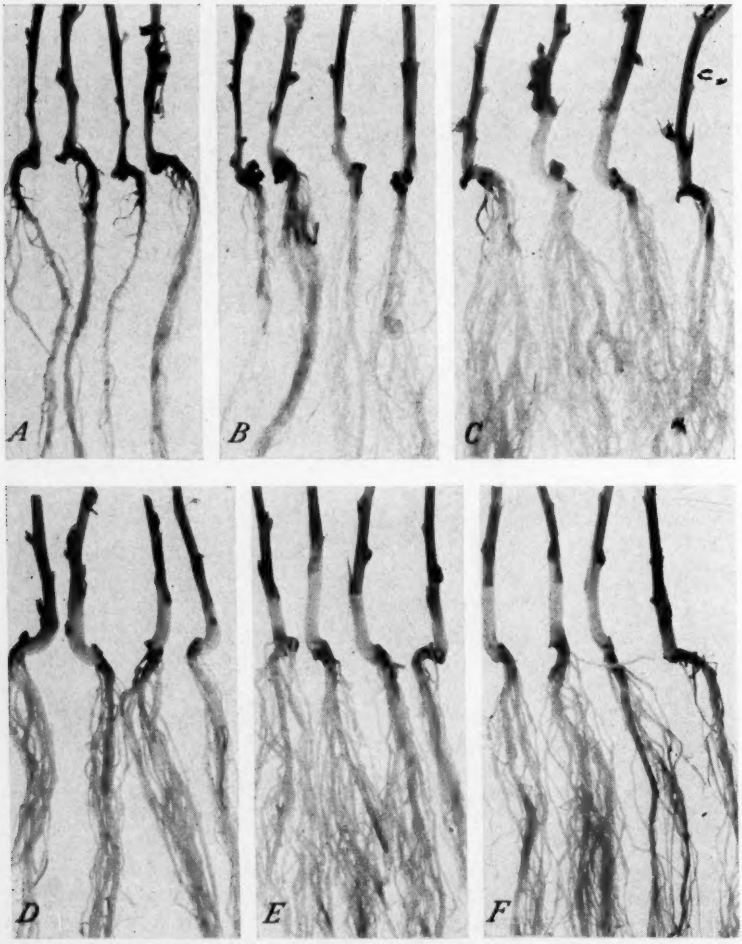

FIGURE 5.—The relation of salt concentration of the nutrient solution to the development of *Aphanomyces* root rot of peas grown in silica-sand culture. See experiment 3 in figure 4. Photographed 27 days after inoculation. The concentrations used were as follows: *A*, 0.1H; *B*, 1H; *C*, 2H; *D*, 3H; *E*, 4H; *F*, 5H (see further explanation in the text). Plants in *A* are severely diseased; many fibrous roots have been killed and cortical decay of the upper taproot and the lower stem is evident. In *B* root decay is moderate and there is considerable cortical decay in the stems. In *C* decay in the root and stem is slight. In *D* there is very slight root and stem discoloration. In *E* and *F* no signs of disease were found.

same, the possibility that one of the major elements was the effective agent still existed. In order to determine whether this was the case, each of the three elements N, P, and K was varied in the same proportions as in the experiments in which the low concentration was used. This was done at each of the three concentration levels 1H, 2H, and 3H. With this range any effect of a single element should be detected readily. With the exception of the experiment with varying nitrogen at the 1H level, a 0.1H check was run with each experiment for purposes of comparison. The results are shown in table 5.

TABLE 5.—*The effect of varied proportions of nitrogen, potassium, and phosphorus at different nutrient concentration levels on the development of Aphanomyces root rot of pea, as indicated by the disease index*

Relative level of ion being varied	N varied at concentration [1]—			K varied at concentration [1]—			P varied at concentration [1]—		
	1H	2H	3H	1H	2H	3H	1H	2H	3H
High	86.0	3.4	3.1	39.1	3.4	0.7	33.7	4.9	0.0
Normal	61.2	3.2	.6	41.0	3.2	.6	36.4	3.2	.6
Minus	77.2	3.3	.7	43.8	8.0	.7	35.6	0	0
0.1H control	.....	82.1	82.1	75.0	82.1	81.4	84.4	82.1	81.4

[1] See text for explanation of symbols indicating nutrient-solution concentration.

On the basis of the previously discussed field experiments made by other investigators, and the one made by the authors, it was expected that the quantity of nitrogen in the nutrient would have considerable influence on the severity of the root rot. The results of these experiments indicate clearly that the variations in the amount of nitrogen used, from none to 15 percent more than in the normal solution, had little or no effect on the degree of infection at any of the three nutrient concentrations. At each concentration level, nitrogen-deficiency symptoms were clearly visible in the series lacking nitrogen before the experiments were terminated, eliminating the possible influence of nitrogen carried in the seed. Since the disease development was so rapid in the lower nutrient levels, there seems to be no reason to believe that the food stored in the seed had any effect on the results of any experiment.

Although the field experiments have shown very little reduction in root-rot damage by the potassium and phosphorus portions of the fertilizers, these two elements were tested in sand culture at the same nutrient concentrations as the nitrogen portion. The results failed to show any influence of high or low amounts of either element as compared with the normal solution on the amount of disease at any of the three nutrient concentrations (table 5).

At the three nutrient levels used in the experiments on elements in varied proportions the same general decrease in disease occurred with increased concentrations, as shown in the experiments involving increased concentrations of the normal nutrient solution. However, it must be kept in mind that at any given nutrient concentration, the total salt concentration of the nutrient solutions remained essentially constant, regardless of the lack or excess of any one element. It is evident, from the results of this set of experiments, that development of the disease is not dependent upon an adequate nutrient balance,

but that the reduction in disease is directly correlated with the increased concentration of the nutrient salts in the root zone of the host plants.

White and Ross (*21*) in a study of the effect of fertilizer salts have found the nitrogen components of present-day fertilizers to be much more active in raising the salt concentration of the soil solution than equivalent amounts of salts of potassium and phosphorus. It seems probable, then, that the favorable action of the nitrogen-bearing fertilizers in reducing root rot in the field is due to the greater activity of the nitrogen fractions of the fertilizer in increasing the salt concentration of the soil solution.

In order to determine the osmotic pressure of the various nutrient concentrations used, the freezing point depression method was employed. A Drucker-Burian microthermometer was used, and with the equation $P=12.06\triangle-0.021\triangle^2$, the osmotic pressure, P, in atmospheres was calculated.[3] The values obtained are shown in figure 4. It is possible that these values could be used as a guide in soil experiments designed to determine the fertilizer requirements for field control of root rot.

The results of the above experiments substantiate the suggestion made by workers at the New Jersey Agricultural Experiment Station (*11, Rpt. 59*) that the increased fertilizer concentration of the soil was responsible for reduced disease when fertilizers were applied. The observations made by Walker and Musbach (*20*) that less root rot developed when a complete fertilizer was placed with the seed than when it was placed away from the seed at the time of planting would seem to be explained by the fact that a greater fertilizer concentration is present at the zone of major root development when the fertilizer is placed with the seed.

EFFECT OF INCREASED NUTRIENT CONCENTRATION AFTER INOCULATION

The investigators at the New Jersey Station (*13, Rpt. 55; 11, Rpts. 56, 57, 59; 14, Rpt. 58*) have found that fertilizers must be applied before infection has occurred in order to be effective in reducing root rot damage. An experiment was carried out to determine the effect on incidence of root rot of increasing the salt concentration from a low to a high level at definite intervals of time after inoculation. The purpose was to find the length of time over which infection takes place under ideal conditions in the sand culture, and to observe whether the increased concentration of nutrients would decrease disease development after infection had taken place. Eight rows of four pots each were started with a normal solution at the 0.1H concentration and one row at the 3H concentration. Two weeks after planting, three pots in each row were inoculated while the fourth was left uninoculated. One row of the 0.1H concentration and the one at the 3H concentration were continued throughout the experiment. Plants in a second row of 0.1H concentration were removed periodically to determine progress of the disease. Of the other six rows, the first was changed to 3H 1 day after inoculation, the second after 2 days, etc. When the 3H solution was applied, all pots in the row were flushed with this solution in order to bring the concentration of

[3] This equation is from Gortner (*4, pp. 317–336*): $\triangle=\triangle'-0.0125\triangle' U$, when $\triangle'$ is the difference in ° C between the freezing point of the solution and that of pure water, while U represents the difference in ° C between the freezing point and the point of undercooling at which crystallization occurs.

the nutrient in the pots to the high level immediately. The results are shown graphically in figure 6.

Daily inspection of the roots of plants in the 0.1H solution disclosed no visible signs of infection until 4 days after inoculation. However, delay of the rise in nutrient concentration for 2 days after inoculation resulted in a marked increase in infection, while delay until the fourth day resulted in little reduction in disease. Thus the shift in concentration of nutrient to a level that would otherwise prevent severe disease development was of no avail if made after infection had occurred. The results of this experiment are in agreement with the field observations at the New Jersey Station, in which

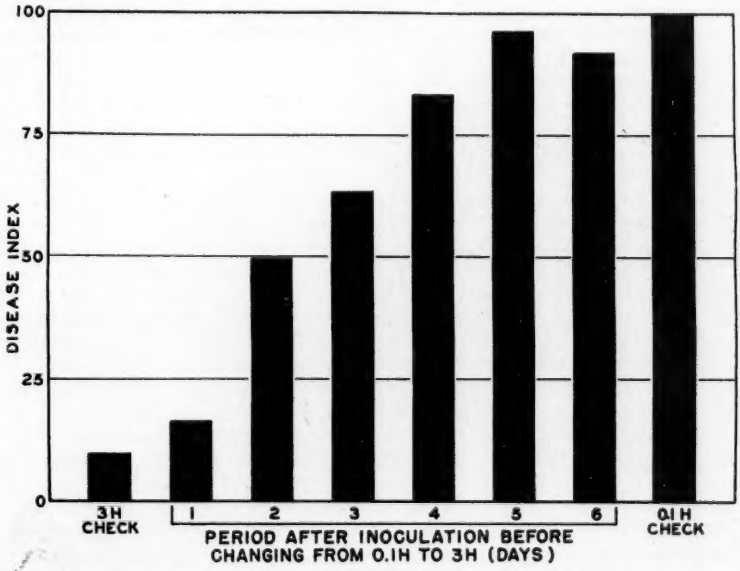

FIGURE 6.—The development of root rot (in terms of disease index) in plants grown at high (3H) and low (0.1H) concentrations of nutrient solution, compared with those started at 0.1H and shifted to 3H at various intervals after inoculation. Note the steady increase in the disease index as the interval between the inoculation and the shift to the high-concentration nutrient was increased.

the beneficial effects of fertilizer did not prevail if the fertilizer was applied after infection had taken place (*13, Rpt. 55*).

EFFECT OF NUTRIENT CONCENTRATION ON GROWTH OF THE FUNGUS

The direct inhibiting effect of high salt concentrations of the nutrient solution on the growth of the organism would seem to be a possible explanation for decreased severity of the root rot disease. In order to determine the effect of the nutrient concentration on the growth of the fungus, agar plates of the normal solutions at the 0.1H, 1H, 2H, 3H, 4H, and 5H levels were poured and inoculated with the fungus. The agar was prepared by making up the nutrient at double strength

and combining with an equal volume of 4 percent agar. The ingredients were sterilized separately and mixed just prior to the pouring of plates. At the higher concentrations 3 to 4 drops of M/1 NaOH per 200 cc. of agar solution were added when necessary to bring the reaction to pH 6.0 or 6.2. The separate sterilization of agar and nutrient solutions was found necessary when previous attempts to sterilize them after mixing had resulted in a strongly acid reaction in the more concentrated nutrients.

The plates were inoculated in the center by 2-mm. disks of agar taken from the periphery of a vigorously growing 4-day-old culture of *Aphanomyces euteiches*. Six plates were used at each nutrient concentration. Daily measurements of radial growth were made. Because of the absence of all organic and other nutrient materials, except for that occurring in the agar, growth was slow and sparse. Nevertheless, growth did occur at the higher nutrient concentrations at approximately the same rate as in the lower, except at the 5H level at which slightly reduced growth was observed. From these results it is apparent that the growth of the organism is not inhibited at the nutrient concentrations at which no infection occurred in the sand cultures.

Geach (*3*) made a brief study of the growth of *Aphanomyces euteiches* in cultures containing various nitrogen compounds. He found growth of the organism to be retarded on pea-decoction agar containing any one of the following compounds: 0.5 percent urea, 1.1 percent NH_4NO_3, and 1.4 percent $NaNO_3$. On Shear's corn-meal agar containing 2.8 percent $NaNO_3$ no growth occurred. The apparent contradiction between these results and those of the authors is readily explained when the total salt concentrations of the sand-culture nutrient solutions and the agar cultures employed by Geach are noted. The osmotic values for the different concentrations of nutrient solutions are shown in figure 4. The calculated osmotic values for the 1.4 percent $NaNO_3$ in the pea-decoction agar used by Geach is approximately 6.8 atmospheres. This is roughly twice the osmotic value of the 5H solution employed in the sand-culture studies, and far in excess of that at which disease control was obtained. Evidently the salt concentration of the 2.8 percent $NaNO_3$ was sufficiently high to prevent growth of the organism. From the previously discussed nutrient studies, it has been shown that the presence or absence of nitrogen has no effect on the severity of root rot. Thus the inhibitory action of nitrogen in some form, as implied by the work of Geach, cannot be considered as the true cause for the control of pea root rot.

DISCUSSION

The *Aphanomyces* root rot of pea is a widespread and often destructive disease of this crop. Up to the present time, the environmental relations and disease control studies have been confined, for the most part, to field observations and experiments. Disease development is favored by high soil moisture, and occurs within a temperature range of about 10° to 30° C. Studies on the control of this disease, made largely by Haenseler at the New Jersey Agricultural Experiment Station and confirmed by other investigators, have shown that applications of complete fertilizers to infested soil tend to reduce

root rot. It has been suggested that the nitrogen fraction in the fertilizer is the most important one in the reduction of disease.

The root rot organism in potato-dextrose-agar culture grows at temperatures from 12° to 32°, inclusive, with an optimum at 28°, while the disease develops in plants grown in inoculated sand maintained at constant temperatures from 16° to 32° with an optimum at 24° and 28° C. No infection was found at 12°, although Jones and Drechsler (*9*) with experiments in soil, involving a longer period of time, found a small amount of infection at 10°. The rate of disease development in sand and the rate of growth of the organism on agar approximate each other quite closely at the various temperatures used.

In naturally infected soil practically no root rot occurred when the moisture was maintained at 45 percent of the water-holding capacity of the soil, while at 75 percent of the water-holding capacity 72 percent of the plants were infected. The soil-moisture relationship found here is in agreement with the observations of other investigators. Since the experiment was not terminated until a month after planting, it would appear probable, under certain field conditions, that a single or an occasional heavy rain would not result in complete root rot infection, but rather that a prolonged condition of high soil moisture is required to produce this condition. This, of course, could happen in poorly drained fields, or under conditions of frequent heavy rainfall, as was the case in Wisconsin in 1940 when root-rot damage was widespread. This is further supported by the results of the 1940 fertilizer trial, for, on June 12, 35 days after planting, 37.8 percent of the plants in the unfertilized check plots were still free from root rot, although rainfall had been abundant. On June 24, after a 12-day period of almost continuous rainfall, all plants were infected, but 6.8 percent were still classed as slightly diseased.

During 1940 the fertilizer trial, mentioned above, was made on a field known to be heavily infested with the root rot organism. Applications of 2–12–6 fertilizer were made at various rates up to 1,108 pounds per acre. One treatment of 0–12–6 at 1,094 pounds per acre was also included. The two highest applications of 2–12–6 (760 and 1,108 pounds per acre) resulted in significantly less disease than in the unfertilized plots. Lesser rates of 2–12–6 and the 1,094-pound rate of 0–12–6 were ineffective in decreasing root rot damage. Since the phosphorus and potash content of the two treatments (2–12–6 at 1,108 pounds, and 0–12–6 at 1,094 pounds) were the same, it is evident that these two components are less active in reducing root rot infection than is the nitrogen fraction. The rainfall during this season was extremely heavy; had there been less rain, it is probable that the degree of control would have been greater in the plots receiving nitrogen in the fertilizer.

When the nutrient solution was used in continuous-flow sand-culture experiments at the concentration described by Hoagland and Snyder (*7*), and in concentrations up to five times that described, infection and disease severity decreased in direct proportion to the increase in the concentration of the nutrient solution. When severe infection occurred in the one-tenth dilution, only a small amount occurred at three times the basic concentration, and none whatsoever

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occurred when the nutrient concentration was raised to four and five times the basic concentration.

At the basic concentration and at two and three times that concentration, solutions each having N, P, and K in excess, and lacking each of these elements were tested. The total salt concentration was maintained constant at each concentration level. The same relation as previously described was observed. The disease decreased as the total salt concentration of the nutrient solution increased; the favorable or unfavorable balance of any of the three essential elements in the nutrient solution in no way modified the disease response.

This apparent disagreement with the results of the fertilizer investigations appears to be explained when the effects of the various fertilizer fractions on the concentration of the soil solution are investigated. White and Ross (*21*), as a result of their study of the effects of fertilizers on the salt content of the soil solution, have found that the nitrogen compounds $NaNO_3$, $(NH_4)_2SO_4$, and NH_4NO_3 increase the salt concentration of the soil solution much more than corresponding quantities of the potassium and phosphorus salts now used in commercial fertilizers. If this is the case, the greater degree of control obtained with nitrogenous as compared with nonnitrogenous fertilizers could probably be attributed directly to the greater increase in the salt concentration of the soil solution by the nitrogen fractions of the fertilizers than by the potassium and phosphate fractions. The possible detrimental effect of nitrogen alone on the organism is ruled out for the reason that the disease development was equally inhibited in nutrient solutions of high salt concentration whether nitrogen was present in excess or lacking entirely.

Under favorable conditions for infection in the sand culture, all plants may become infected within 5 days after inoculation. After infection has occurred, and before any but the slightest macroscopic symptoms are visible, changing the solution concentration from a low level to one sufficiently high to largely inhibit infection, if done at the outset, does not delay the development of the disease within the host plant. For this reason it would appear that the action of the high concentration of the nutrient salts is that of preventing infection, either by direct action of some kind on the organism, or by rendering the host more resistant. In the latter case the promotion of host resistance is much less pronounced after infection has taken place.

On agar made from nutrient solutions ranging from one-tenth to five times the basic concentration, the organism appeared to grow equally well. Only a slight depression in growth rate was noted at the highest nutrient concentration. Therefore it appears likely that the mechanism by which the root rot is prevented when in the presence of high salt concentrations is due to some other cause than concentrations of nutrient salts inhibitory to the organism.

A possible explanation for the lack of root rot at the high nutrient concentrations is to be found in the work of Nightingale and Farnham (*15*). In a study of the effect of the nutrient concentration on the anatomy of the sweet pea (*Lathyrus odoratus* L.) they have found the roots of plants growing in dilute solutions to be highly succulent and lacking in mechanical strength. The roots of plants grown in high nutrient concentrations, on the other hand, were typically woody, mechanically strong, and distinctly lacking in succulence. A similar condition occurred in the stem tissue. Since the range in nutrient

concentration employed by them (0.5 to 3.0 atmospheres osmotic pressure) closely paralleled that used in the present investigations (0.12 to 3.5 atmospheres), it may possibly be, in the case of pea root rot, that the host plants acquire a morphological resistance as a direct result of the high concentration of the nutrient when grown in such solutions.

SUMMARY

The investigations comprise a study of temperature, moisture, and nutrition in relation to root rot (*Aphanomyces euteiches* Drechs.) of peas.

On potato dextrose agar the most rapid radial expansion of the organism occurred at 28° C. No growth occurred at either 8° or 36°. The optimum temperature for disease development on plants grown in sand was found at 24° and 28°. No infection was noted at 12° during an 11-day period, while nearly all plants at the optimum temperature were severely affected in that period.

On phosphate-buffered potato-dextrose agar, the pH limits for growth were about pH 3.4 and slightly above pH 8.0. The optimum, as measured by radial expansion of the organism, occurred between pH 4.5 and 6.5. An apparent isoelectric point appeared at pH 5.9.

In infested soil, practically no infection occurred when the moisture was maintained at 45 percent of the water-holding capacity. At 75 percent of moisture-holding capacity, infection was quite severe.

Under conditions of controlled nutrition in a continuous-flow sand culture, inoculated by means of a zoospore suspension, the severity of disease decreased in direct proportion to the increase in total salt concentration of the nutrient solution. No infection occurred at the highest concentrations employed, while all plants were severely diseased at the lowest concentration. Varying the ratio of each of the elements, N, P, and K from complete absence to an excess of that in the balanced solution had no effect on disease development, whether in dilute or concentrated nutrient solutions.

When conditions are favorable in the sand culture, all plants may become infected within 5 days. Once infection takes place, high nutrient concentrations do not appear to inhibit the development of the disease.

On agar cultures made from the nutrient solutions employed in the sand culture, the organism grew readily on the concentrations at which infection was prevented in the sand culture.

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INHERITANCE OF A MELANINLIKE PIGMENT IN THE GLUMES AND CARYOPSES OF BARLEY [1]

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INTRODUCTION

Although black barleys are rarely grown commercially in the United States, they have been used in hybridization for many years. Many valuable characters, including smooth awns, have been transferred from varieties with black hulls and caryopses. A large number of varieties and strains of black barley have been introduced or selected from crosses involving black and white color. The factor pair responsible for the production of black pigment in barley has been studied by a number of workers, but no reference has been made to the various degrees of black coloration that exist. This paper presents the results of a study of the inheritance of the black or melaninlike pigment that occurs in various intensities in the glumes and caryopses of barley (*Hordeum* spp.) seed in some 8,000 F_3 progenies from more than 50 crosses.

REVIEW OF LITERATURE

The character black vs. white flowering glumes has been reported to be controlled by a single factor pair in several publications reviewing genetic studies in barley. Black has been considered completely dominant over white, giving F_2 ratios of 3 black to 1 white. Tschermak (*13*)[2], Biffen (*1*), Griffee (*4*), Hayes et al. (*6*), Ubisch (*14*), Hor (*7*), Robertson (*10*), Sigfussen (*12*), Buckley (*2*), and Kuckuck (*9*) have all reported monogenetic ratios of black vs. white caryopses or glumes. Harlan (*5*) reported the black color to be the result of a melaninlike pigment found in the lemma, palet, and pericarp only, and the color in the aleurone to be due to anthocyanin pigments. A special study of color inheritance in barley and possible linkage relationships has been reviewed and discussed by Buckley (*2*). He concluded that black pericarp is always associated with black lemma, that either the same gene is responsible for the coloring in both the lemma and the pericarp or that very close linkage between two separate genes must exist. No linkage was found between the genes controlling black vs. white glume and pericarp color and any of the anthocyanin factors. The genes for black and white color were reported by Buckley to be inherited independently when tested with long vs. short rachilla

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[2] Italic numbers in parentheses refer to Literature Cited, p. 28.

hairs, hooded vs. awned, and hulled vs. hulless. Gaiser (*3*) had previously reported that black lemma was linked with the texture of awn, rachilla hairs, color of caryopsis, one albino factor, and one factor for the resistance to *Helminthosporium*. Hor (*7*) reported black lemma linked with texture of awn and length of rachilla hairs. Buckley (*2*) found three linkage groups and in addition suggested that the factor pairs black vs. white and hulled vs. naked caryopsis represented two additional linkage groups. More recent work by Robertson, Wiebe, and Immer (*11*) indicates that the factor pair black vs. white is in linkage group No. 2 along with $A_t a_t$, an albino factor found in Trebi I. They list a factor responsible for the production of a third outer glume in the same linkage group with black vs. white lemma. Ivanova (*8*) describes this third outer glume as a new character in barley. Seed of this particular barley has not been available to workers in the United States, so that the genes controlling black vs. white and $A_t a_t$ are the only genes in linkage group No. 2 available to them.

Sigfussen (*12*) has pointed out that the black pigment does not express itself until just before ripening, making it difficult to classify immature plants.

No reference was found in the literature to studies of crosses between colored parents that differed in the intensity of black pigment in the lemma and caryopsis.

MATERIAL AND METHODS

A number of varieties and strains containing anthocyanin and the melaninlike pigment were used in this study.[3] A few white-glumed varieties thought by some workers to produce occasional dark types also were included in an observational nursery. These varieties have been grown for periods of 7 to 9 years. Head selections also have been made from them, some of which appeared to show greater color variation than others.

After preliminary studies all of the varieties were arbitrarily classified into five groups based on the intensity of the melaninlike pigment. Two of the varieties in each group breeding most uniformly for color intensity were chosen for the genetic studies. These groups were designated as (1) dense black, (2) black, (3) medium black, (4) gray, and (5) white.

Although the climatic conditions were generally favorable for color classification, it was difficult in some crosses. Attempts were made to extract the black pigment so that the extracts might be used as a basis for classifying color, but with no success.

Most of the more than 100 black-glumed varieties and strains were found to be relatively stable and uniform for color intensity. A few varieties such as Blackhull had previously been reported to contain some individuals with lighter colored glumes and caryopses than the variety as a whole. In a study of a large number of head rows of Blackhull (C. I.[4] 878), a light-colored type was isolated and found to breed true. This light-colored strain of Blackhull (C. I. 878) was used as one of the parent strains in the gray group. A number of the color variants found in the original Blackhull variety were definitely

[3] Obtained from H. V. Harlan, principal agronomist, in charge of barley investigations, Division of Cereal Crops and Diseases, Bureau of Plant Industry.

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

identified as mixtures or hybrid segregates. A few other varieties, including C. I. 875 and 3204, contained two or more color classes which, when isolated, have continued to breed true for dark and light glumes, respectively. There is no evidence from a 9-year study that any of the varieties grown under Utah conditions have thrown mutants of either lighter or darker color intensity than was found in the original material after pure color types were established.

The varieties used in this study to represent the five color-intensity groups are shown in table 1, and some of them are illustrated in figure 1. Group 1, arbitrarily classed as dense black, is characterized by its

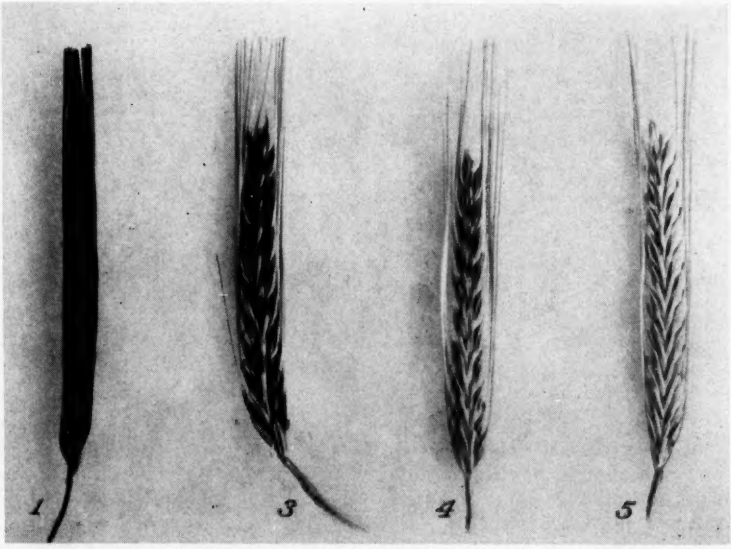

FIGURE 1.—Spikes of barley illustrating four of the five color-intensity groups: Group 1, dense black (*BB*); group 3, medium black ($B^{mb}B^{mb}$); group 4, gray ($B^{g}B^{g}$); and group 5, white (*bb*).

extreme black pigmentation in the flowering glumes, pericarp, and awns. Blackhull (C. I. 878) and Black Algerian (C. I. 708) are representative of this group. Group 2, classed as black, appeared to be slightly lighter in color intensity than group 1, the awns often showing a yellowish color. Bucher (C. I. 1461), Donjon (C. I. 1264), an unnamed variety (C. I. 3910–1), and Jet (C. I. 2222) are listed as representative varieties in group 2. Group 3, described as medium black, shows slightly less pigmentation than group 2. The lateral florets are generally light-colored in the two-rowed and intermediate segregates found in this group. C. I. 4376 and 2970 are representative varieties. Group 4 is best described as gray in color and generally shows sufficient contrast to the other groups to be easily identified. A light-colored type isolated from Blackhull (C. I. 878) and Dentil (C. I. 1260) was chosen to represent this group. In group 5 are included varieties with white glumes and pericarp, of which C. I. 4354 and Blackhull 1178 (C. I. 5679) are representative.

All combinations among 2 of the above varieties from each of the 5 color classes were made. The total of 45 crosses involved combinations of all fertility classes such as two-rowed, six-rowed, intermedium, and deficiens, as well as other character differences. Other crosses also were made for additional information on the inheritance of black color.

TABLE 1.—*Color-intensity groups and descriptions of the varieties selected to represent them*

Group and variety	C. I. No.	Varietal characters			
		Glume and pericarp color	Awn color	Fertility type of lateral florets	Caryopsis
1, Dense black:					
Blackhull	878	Dense black	Black	Deficiens	Hulled.
Black Algerian	708	do	do	Vulgare	Do.
2, Black:					
Jet	2222	Black (lateral florets gray).	Dark to light	Intermedium	Hulless.
Bucher	1461	Black	do	Vulgare	Hulled.
Unnamed	3910–1	Brown to black	do	do	Hulless.
Donjon	1264	Black	do	do	Hulled.
3, Medium black:					
Unnamed	4376	Dilute black	Gray	Deficiens	Do.
Do	2970	Dilute black (laterals yellow).	do	Distichon	Do.
4, Gray:					
Blackhull (light-colored).	878	Gray	Colorless	Deficiens	Do.
Dentil	1260	do	do	Distichon	Do.
5, White:					
Unnamed	4354	White	do	Vulgare	Do.
Blackhull selection 1178.	5679	do	do	Intermedium	Do.

Experience indicated that observations on the primary spikes were more reliable than those on spikes chosen at random and that all material should be thoroughly ripened before harvest in order to classify color density accurately. This latter fact also was pointed out by Sigfussen (*12*).

All crosses were studied through the F_3 generation as a check on F_2 classifications. Numerous doubtful types were continued in head rows for 2 or 3 years longer or until they could be identified more positively. Normal color densities failed to develop in seasons of excessive moisture or cloudy weather. Such seasons sometimes necessitated the study of large populations in the F_4 and F_5 generations in order to determine the breeding behavior of the F_2 plants.

The material was space-planted in rows 8 to 17 feet long. A large part of the material was studied in the field.

EXPERIMENTAL RESULTS

COLORED × WHITE

Pigmented varieties representing the four color-intensity groups were crossed with varieties having white or colorless glumes and pericarps. In each case F_2 ratios of 3 black to 1 white resulted, as is shown in table 2. This is in agreement with the ratios generally obtained by previous workers. No intermediate color classes could be found, regardless of the intensity of the black parent used.

TABLE 2.—*F_2 segregations in crosses between colored and white varieties*

Cross	Genetic symbols	F_2 segregation based on F_3 breeding behavior		Deviation / Standard error
Dense black × white	$BB \times bb$	Dense black	White	
Blackhull × Blackhull selection 1178		230	75	0.17
Blackhull × C. I. 4354		340	113	.03
Black Algerian × C. I. 4354		211	80	.97
Black Algerian × Blackhull selection 1178.		156	64	1.40
Black × white	$BB \times bb$	Black	White	
C. I. 3910–1 × C. I. 4354		527	185	.61
Bucher × C. I. 4354		205	71	.28
Jet × C. I. 4354		353	124	.50
Donjon × C. I. 4354		110	46	1.29
C. I. 3910–1 × Blackhull selection 1178		257	93	.68
Jet × Blackhull selection 1178		153	72	2.43
Donjon × Blackhull selection 1178		51	21	.82
Medium black × white	$B^{m\,b}B^{m\,b} \times bb$	Medium black	White	
C. I. 2970 × C. I. 4354		262	99	1.25
C. I. 2970 × Blackhull selection 1178		194	83	1.91
Gray × white	$B^{g}B^{g} \times bb$	Gray	White	
Blackhull gray segregate × C. I. 4354		216	79	.71
Dentil × C. I. 4354		253	96	1.08
Dentil × Blackhull selection 1178		326	126	1.41
White × white	$bb \times bb$	No segregation		
Blackhull selection 1178 × C. I. 4354				
Total		3,844	1,427	

It should be noted that the white segregates are in excess in all but two of the crosses. In all crosses studied the deviation in favor of light-colored segregates is still more pronounced, giving 6,166 dark to 2,234 light, with a deviation of 134 ± 40. No satisfactory explanation has been found for this behavior.

COLORED × COLORED

Tabulated results of the segregations from crosses of colored × colored are shown in table 3.

DENSE BLACK × DENSE BLACK

No indication of any segregation could be observed in the F_2 and later generations of crosses between dense black and dense black varieties.

DENSE BLACK × BLACK

Six crosses of dense black × black were studied, but classification was difficult. These two color groups differ so slightly in intensity of coloration, which expresses itself largely in the awns, lateral florets, and culms, that they may be included in one group.

DENSE BLACK × MEDIUM BLACK

With favorable seasonal conditions the segregating generations from the cross of dense black × medium black could be classified. A single-factor difference explains the results obtained. Varieties or segregates of the six-rowed type consistently have a slightly lighter coloration than those of the non-six-rowed types. When the color classes of the parents were similar, classification of the segregates was more difficult in the progeny from crosses involving six-rowed by non-six-rowed varieties than when both parents were of like fertility.

TABLE 3.—*F_2 segregations in crosses between pigmented varieties representing the different color-intensity groups*

Cross	Genetic symbols	F_2 segregation based on F_3 breeding behavior		Deviation / Standard error
Dense black × dense black	$BB \times BB$			
Blackhull × Black Algerian		No segregation		
Dense black × black	$BB \times BB$			
Blackhull × Bucher		No segregation		
Blackhull × Jet		do		
Black Algerian × Jet		do		
Black Algerian × C. I. 3467		do		
Blackhull × C. I. 3910–1		do		
Black Algerian × C. I. 3910–1		do		
Dense black × medium black	$BB \times B^mbB^mb$	Dense black	Medium black	
Blackhull × C. I. 2970		176	72	1.47
Black Algerian × C. I. 2970		Difficult to classify		
Dense black × gray	$BB \times B^gB^g$	Dense black	Gray	
Blackhull × C. I. 1260		215	80	.84
Black Algerian × Blackhull gray segregate.		176	70	1.25
Black Algerian × Dentil		74	23	.29
Black × black	$bB \times BB$			
Donjon × C. I. 3910–1		No segregation		
Jet × C. I. 3910–1		do		
C. I. 4363 × Jet		do		
Jet × Bucher		do		
Black × medium black	$BB \times B^mbB^mb$			
Jet × C. I. 2970		Difficult to classify		
C. I. 3910–1 × C. I. 2970		do		
Black × gray	$BB \times B^gB^g$	Black	Gray	
Bucher × Blackhull gray segregate		187	67	.51
C. I. 3910–1 × Blackhull gray segregate.		228	87	1.07
Donjon × Blackhull gray segregate		125	43	.18
C. I. 3467 × Dentil		166	42	1.60
Bucher × Dentil		188	57	.63
C. I. 3910–1 × Dentil		215	68	.38
Medium black × medium black	$B^mbB^mb \times B^mbB^mb$			
C. I. 2970 × C. I. 4376		No segregation		
Medium black × gray	$B^mbB^mb \times B^gB^g$	Medium black	Gray	
C. I. 2970 × Blackhull gray segregate		305	117	1.29
C. I. 4376 × Dentil		94	28	.52
C. I. 2970 × Dentil		173	53	.54
Gray × gray	$B^gB^g \times B^gB^g$			
Blackhull gray segregate × Dentil		No segregation		

DENSE BLACK × GRAY

Crosses involving dense black × gray were extremely clear-cut in their segregation. A monofactorial condition was suggested by all such crosses.

BLACK × BLACK

No indication of segregation was observed in any of the four crosses studied involving black × black. In C. I. 3910–1, a hulless variety, the pericarp was more densely colored at full maturity than the glumes.

BLACK × MEDIUM BLACK

Differences in the parents chosen for black and medium black could be seen when conditions for color development were favorable. Crosses involving these two groups, although indicating segregation, did not permit clear-cut separation in the F_2 or later generations.

BLACK × GRAY

Each of the six crosses studied indicated a single-factor difference between the color intensities black and gray. In seasons when bleaching was abnormal it was necessary to continue considerable material into the F_4 or F_5 generation in order to separate the classes.

MEDIUM BLACK × MEDIUM BLACK

No segregation was evident when two varieties of this color-intensity group were crossed.

MEDIUM BLACK × GRAY

Crosses between varieties of the medium black and the gray classes yielded monofactorial segregations in the F_2 generation.

GRAY × GRAY

A cross between C. I. 1260 × Blackhull (C. I. 878) (gray) showed no indication of segregation in later generations.

DISCUSSION

Although five color-intensity groups were described and studied, the problem would have been simplified if the group listed as black had been eliminated or combined with dense black. Great difficulty was encountered in classifying the plants in crosses involving black × dense black or black × medium black. It appears that the black group may differ slightly from either of these other dark-colored groups and that possibly still other color groups might be found by a positive method of determining color intensity. The medium-black group appears to be distinguishable from the other groups but is not too well established as yet.

Using only data from crosses in which the segregates could be classified by inspection, the results definitely establish three color groups, the dense black (*BB*), gray (B^gB^g), and white (*bb*). The factors responsible for the production of the melaninlike pigment appear to represent an allelomorphic series, since only monofactorial ratios were obtained from any combination showing color-intensity differences. In all crosses the darker color class showed complete dominance.

These studies also suggest that certain other varieties might give more clear-cut results in crosses.

The Dentil variety displayed a winter-grown habit in two seasons, which complicated the studies, but early seeding eliminated further difficulty in this respect.

Buckley (*2*) concluded that black pericarp is always associated with black lemma and that either the same gene is responsible for the coloring or close linkage exists. No segregates exhibiting recombinations of glume and pericarp colors were obtained in these experiments.

SUMMARY

No mutation or new off-color type was observed during a study of more than 100 varieties and strains of barley, although in some varieties individual plants segregated for two color classes.

Barley varieties were grouped into five color classes, based upon the intensity of the black melaninlike pigment in the glume and pericarp. These groups were (1) dense black, (2) black, (3) medium black, (4) gray, and (5) white. More than 50 crosses involving all group combinations were made and studied in the segregating generations. Eliminating group 2 (black), all crosses among the remaining four groups yielded monofactorial segregations in the F_2 generation. More refined classification might establish one or more additional color intensities.

The medium-black group appeared to be distinct from the others, but its relationship could not be established.

The data suggest an allelomorphic series of factors causing various color-intensity expressions. The factors definitely established are black (*BB*), gray (B^gB^g), and white (*bb*). The denser color was always completely dominant over the lighter one.

Despite attempts to isolate other color groups from the progeny of certain crosses, the parental types only were recovered in the homozygous condition in later generations.

Pigment formation in both the pericarp and the flowering glume appears to be controlled by a single series of allelomorphic genes.

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A STUDY OF THE C FACTOR AS A REQUIREMENT FOR GROWTH OF HEMOPHILUS GALLINARUM [1]

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INTRODUCTION

The so-called C factor requirement for the growth of *Hemophilus gallinarum* [2] was reported by Kessens,[3] who observed that chicken serum heated at 90° to 100° C. for 10 minutes supported growth of *H. influenzae*, but failed to support growth of the fowl coryza bacillus, *H. gallinarum*.

Delaplane et al.[4] found that some factor in chicken-blood serum was adversely affected by boiling but was not completely destroyed, since some growth occurred when the boiled serum was used at the base of nutrient agar slants. For convenience, they also referred to this factor as the C factor.

Because of the incomplete destruction of the factor in boiled blood serum, the question arose as to the possibility that the physical changes brought about by boiling might have prevented the organism from utilizing some necessary nutrient and that the effect observed was not the result of chemical action.

METHODS

To avoid the firm type of coagulated proteins such as result from boiling serum or diluting serum in broth, it was decided to add the sterile chicken-blood serum in the ratio of 1 part of sterile serum to 3 parts of a sterile 2-percent sodium citrate normal salt solution. Such a solution is alkaline, and it was thought that the metaproteins resulting from boiling would remain in suspension. This proved to be the case, as the serum solution merely became cloudy after boiling or after heating for 15 minutes at 15 pounds' pressure in the autoclave.

Serum solutions thus prepared were boiled for 5, 10, and 15 minutes, rapidly cooled, and used with nutrient broth and at the base of nutrient agar slants for cultural tests. Serum solutions similarly prepared were autoclaved and tested in the same manner. Sterile chicken-blood plasma instead of serum was also used in the tests.

As boiling in the manner described did not appear to destroy the growth properties of the serum or plasma for *Hemophilus gallinarum*, boiled preparations of serum and plasma were centrifuged, the supernatant filtered through filter paper, and the filtrate sterilized by passing through Swinny bacteriological filters.

Unheated serum and plasma filtered in this manner were known to support growth of *Hemophilus gallinarum*, and so were not employed.

[1] Received for publication December 9, 1940. Contribution No. 577 of the Rhode Island Agricultural Experiment Station.

[2] Kessens refers to this organism as *Haemophilus coryza*.

[3] KESSENS, B. H. VERGELIJKEND ONDERZOEK BETREFFENDE HAEMOPHILUS CORYZAE, HAEMOPHILUS INFLUENZAE EN ANDERE HAEMOPHIELE BACILLEN. Rijks Univ., Utrecht. 1936.

[4] DELAPLANE, J. P., ERWIN, L. E., and STUART, H. O. THE EFFECT OF THE X FACTOR, OF SODIUM CHLORIDE, AND OF THE COMPOSITION OF THE NUTRIENT MEDIA UPON THE GROWTH OF THE FOWL CORYZA BACILLUS, HEMOPHILUS GALLINARUM. Jour. Agr. Res. 56: 919-926. 1938.

Two different strains of *Hemophilus gallinarum* and a smooth strain of *H. influenzae* were used for testing the boiled serum and plasma solutions, and a bacteriological filtrate was obtained from them.

RESULTS

The results of the various tests are shown in table 1. The boiled serum and plasma solutions supported good growth of *Hemophilus gallinarum*, whereas the autoclaved preparation failed to do so. This would seem to indicate that boiling the serum or plasma does not destroy the factors necessary for growth; and that the possible destruction of the factor in serum as reported by Kessens [5] and by Delaplane et al.[6] resulted from physical changes in the medium.

The results of filtering the boiled preparations would seem to indicate that the growth factors are tied up with the coagulation proteins since the filtrates failed to support growth.

The tests in which *Hemophilus influenzae* was used showed that it, too, would grow in the boiled citrated serum and plasma preparations, but not in the bacterial filtrates of the same material.

It may be possible that through the action of enzymes or other factors *Hemophilus influenzae*, unlike *H. gallinarum*, is able to utilize the growth factors of the more firmly coagulated forms of the boiled chicken serum proteins (as reported by Kessens [5]), but no studies of this kind have been attempted.

As a result of these studies, the writers believe the C factor of Kessens [5] to be one of a physical rather than of a distinctly chemical or nutrient nature, and thus the cultures of *Hemophilus gallinarum* used in the present work require only the X and V factors for growth rather than the X, V, and C factors.

TABLE 1.—*The effects upon the growth of Hemophilus gallinarum and H. influenzae of different methods of treating preparations of 25-percent chicken-blood serum or or plasma, in 2-percent sodium citrate normal salt solution*

Treatment of chicken-blood preparation and method of use	Type of growth (as indicated by stained preparations)	
	H. gallinarum [1]	H. influenzae
Serum boiled 5 minutes, used at base of agar slants	Good growth	Good growth.
Serum boiled 5 minutes, used with broth	Growth	Growth.
Serum boiled 10 minutes, used at base of agar slants	Good growth	Good growth.
Serum boiled 10 minutes, used with broth	Growth	Growth.
Serum boiled 15 minutes, used at base of agar slants	Good growth	Good growth.
Serum boiled 15 minutes, used with broth	Growth	Growth.
Serum autoclaved 15 minutes, used at base of agar slants	No growth	No growth.
Boiled serum Swinny filtrate at base of agar slants	do	Do.
Plasma boiled 5 minutes, used at base of agar slants	Good growth	Good growth.
Plasma boiled 5 minutes, used with broth	Growth	Growth.
Plasma boiled 10 minutes, used at base of agar slants	Good growth	Good growth.
Plasma boiled 10 minutes, used with broth	Growth	Growth.
Plasma boiled 15 minutes, used at base of agar slants	Good growth	Good growth.
Plasma boiled 15 minutes, used with broth	Growth	Growth.
Plasma autoclaved 15 minutes, used at base of agar slants	No growth	No growth.
Boiled plasma Swinny filtrate at base of agar slants	do	Do.

[1] No differences were noted between the 2 strains of *H. gallinarum* used in the tests.

[5] KESSENS, B. H. See footnote 3.
[6] DELAPLANE, J. P., ERWIN, L. E., and STUART, H. O. See footnote 4.

THE DISTRIBUTION OF POTASSIUM IN BRIGHT LEAF CIGARETTE TOBACCO AND ITS INFLUENCE ON THE QUALITY OF THE LEAF [1]

By M. F. GRIBBINS, *assistant in agricultural and biological chemistry*, J. J. REID *assistant professor of bacteriology*, and D. E. HALEY, *professor of soils and phytochemistry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Numerous workers have reported that potash fertilization of leaf tobacco is favorable both to the yield and quality of the crop. Many fundamental studies have been conducted on the absorption and translocation of potassium by growing plants and its accumulation within the various cells and tissues. The relation of this element to various biochemical and physiological phenomena has received due consideration.

The present investigations involve a study of the influence of various quantities of potash fertilizers applied to growing plants in the field on the absorption and localization of potassium within the plants and the influence of this element upon their quality. For these studies, samples of tobacco were obtained from certain of the experimental plots at Chatham, Va., where the Virginia Agricultural Experiment Station is conducting field tests on this crop. Samples of both the 1938 and 1939 crops were taken in December from a barn where the mature leaves had been stored in the usual manner after harvesting and curing. The results of studies conducted on similar samples of the 1937 crop have been reported (*2*).[2]

REVIEW OF THE LITERATURE

Within the growing plant potassium may be found in the form of compounds which are quite soluble in water. It is readily transported throughout the plant and is usually found to accumulate in regions of active growth. Nightingale, Schermerhorn, and Robbins (*6*) found that the greater part of the potassium in tomato plants was soluble in water and, when present in limited quantities, was translocated from regions of less intensive to regions of more intensive metabolism. If a potassium-deficient plant was allowed to fruit, the potassium was translocated to the fruit at the expense of all other portions of the plant, which resulted in its death. Confirmatory results have been reported by James and Penston (*3*) and by Janssen and Bartholomew (*4*). The last-named workers found that leaf starvation in the tomato plant due to a lack of potassium was progressive, and extended from the lower to the upper leaves. Dead leaves taken from the plants showed a much lower content of potassium than actively growing leaves, indicating a reutilization of this element in metabolism. It is apparent, therefore, that a plant should contain ample quantities of

[1] Received for publication August 31, 1940. Paper No. 990 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 38.

potassium for its normal growth and metabolism, and that there should be a fair distribution of this element throughout the plant.

MATERIALS USED AND METHODS OF ANALYSIS

The samples were taken from plots each of which received 30 pounds of nitrogen, 80 pounds of phosphoric acid, and various quantities of potash per acre as shown in table 1. Supplementary treatments involved the application of magnesia and limestone to all plots at the rate of 200 pounds per acre. Two percent of the potash was applied as muriate, the remainder as the sulfate. Representative samples [3] of leaves were taken from the lower, the middle, and the upper third of the stalks. Each sample represented two grades, good quality and scrap quality. In 1938 only the good quality grades were analyzed.

Methods used for chemical analysis involved the removal of the midrib and grinding the remainder in a Wiley mill. Total nitrogen was determined by the Kjeldahl method modified to include the nitrogen of nitrates, potassium by the platinic-chloride method according to Thomas et al. (*10*), and moisture by drying at 97.5° C. for 2 hours in a drying oven. Reducing materials were determined on an aqueous extract of the sample by the Quisumbing and Thomas method (*7*) and calculated as glucose. Samples were extracted with water at 50° C. for 2 hours and then overnight at room temperature. The samples were clarified, deleaded, made up to volume, filtered, and the reducing power determined on aliquots.

TABLE 1.—*Fertilizer treatment of the experimental tobacco plots studied* [1]

Plot No.	Row treatment, 1,000 pounds per acre of indicated formula	Side dressing of K_2O per acre [2]	Total K_2O in treatment, per acre
		Pounds	*Pounds*
1	3-8-0	0	0
2	3-8-3	0	30
3	3-8-6	0	60
4	3-8-6	30	90
5	3-8-6	60	120
6	3-8-6	100	160
7	3-8-6	140	200
8	3-8-6	240	300

[1] Data presented in this table were obtained from E. M. Matthews, superintendent of the Chatham substation of the Virginia Agricultural Experiment Station.

[2] Side dressings were applied on either side of the row about 8 inches from the plants 3 weeks after transplanting.

PRESENTATION OF DATA

Samples of both the 1938 and 1939 crops were analyzed for potassium, nitrogen, and reducing materials. The reducing materials, to a large extent, are made up of fermentable carbohydrates. A large portion, but by no means all, of these materials is made up of glucose and related sugars.

The 1938 crop produced good yields and commanded a fair price on the market (table 2), notwithstanding the fact that it was grown under conditions of excessive rainfall and was somewhat damaged by hail. The analyses made on the best quality samples are presented in table 3.

[3] These samples were submitted through the courtesy of E. M. Matthews, superintendent of the Chatham substation.

TABLE 2.—*Effect of various fertilizer treatments on the yield and value of the 1938 and 1939 crops of bright leaf tobacco produced on the experimental plots studied*

Plot No.[1]	Yield per acre	Value per acre	Price per pound
	Pounds	*Dollars*	*Cents*
1938 CROP			
1	758	140.20	18.5
2	1,096	218.08	19.9
3	1,168	239.70	20.5
4	1,192	254.35	21.3
5	1,188	233.63	19.7
6	1,222	241.09	19.7
7	1,198	222.74	18.6
8	1,228	237.53	19.3
1939 CROP			
1	888	120.40	13.6
2	1,096	150.80	13.8
3	1,112	155.60	14.0
4	1,104	162.00	14.7
5	1,084	147.60	13.6
6	1,120	156.40	14.0
7	1,148	143.60	12.5
8	1,076	144.80	13.5

[1] See table 1 for formulas and quantities of fertilizers used on these plots.

The 1939 crop was grown under dry conditions early in the season but later the rainfall was satisfactory. On the whole, however, this crop was somewhat inferior to that of the 1938 crop (table 2).

Chemical analyses of the tobacco for both years are presented in table 3, all results being reported on a moisture-free basis. From the results as listed in table 3, the ratio of nitrogen to potassium was calculated. The results are presented in figures 1 and 2.

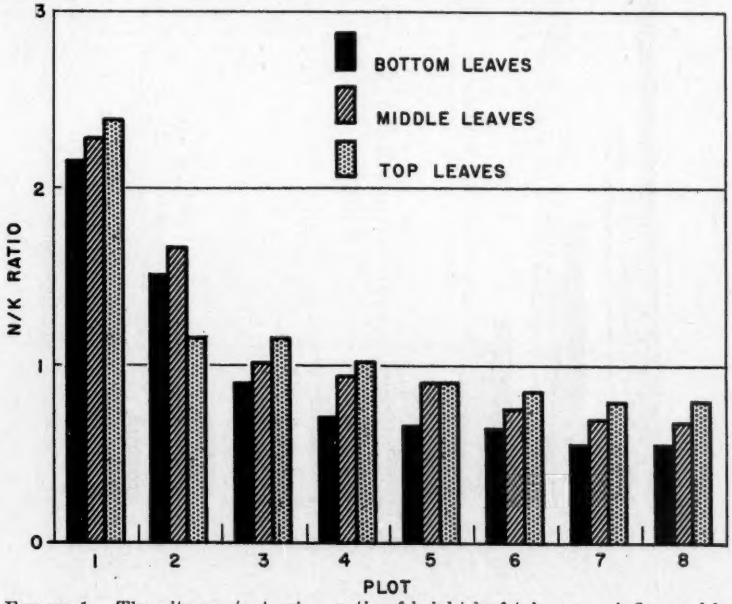

FIGURE 1.—The nitrogen/potassium ratio of bright leaf tobacco as influenced by fertilizer treatments, 1938 crop, best quality.

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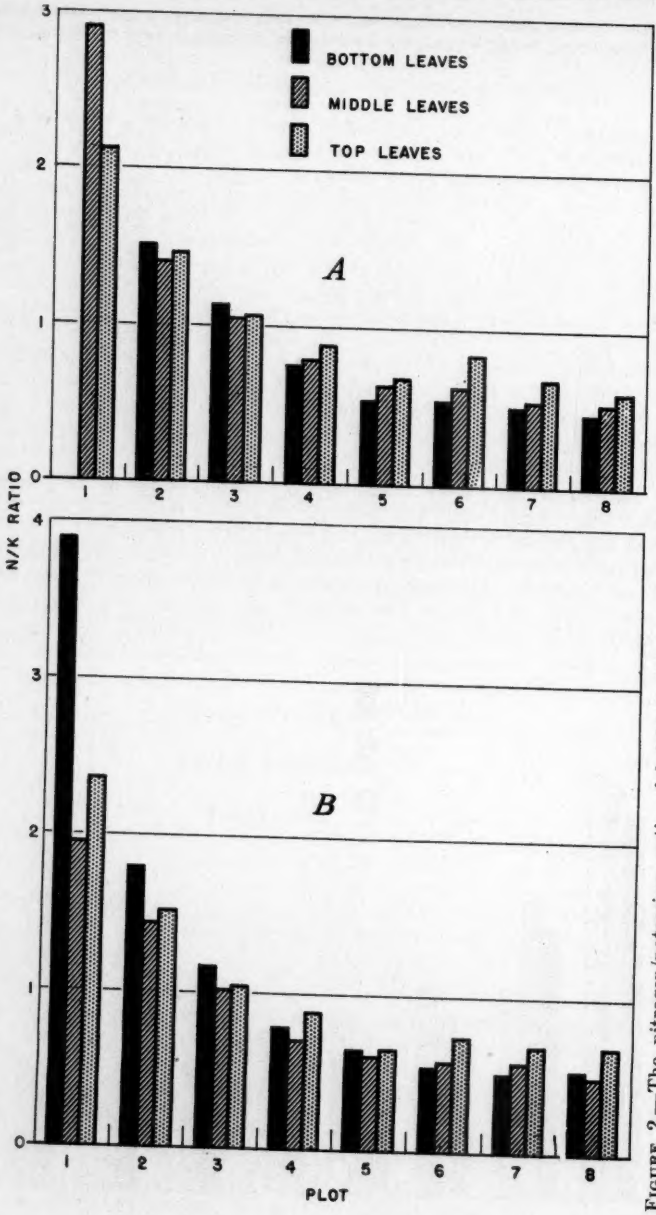

FIGURE 2.—The nitrogen/potassium ratio of bright leaf tobacco as influenced by fertilizer treatments, 1939 crop: *A*, Best quality; *B*, scrap quality.

TABLE 3.—*Effect of various fertilizer treatments on certain constituents of the bottom, middle, and top leaves of best-quality bright leaf cigarette tobacco produced on the experimental plots in 1938, and of best and scrap quality in 1939*

1938, BEST QUALITY

Plot No.[1]	Location of leaves	Reducing materials calculated as glucose	Nitrogen	Potassium	Nitrogen/potassium ratio	Glucose/nitrogen ratio
		Percent	*Percent*	*Percent*		
1	Bottom	22.47	1.97	0.91	2.16	11.40
	Middle	13.48	2.60	1.14	2.28	5.18
	Top	5.87	3.22	1.35	2.38	1.83
2	Bottom	23.65	1.79	1.18	1.52	13.20
	Middle	20.06	2.11	1.26	1.67	9.50
	Top	24.76	2.20	1.92	1.15	6.70
3	Bottom	24.03	1.71	1.91	.90	14.05
	Middle	21.83	1.95	1.93	1.01	11.19
	Top	15.43	2.31	2.00	1.15	6.68
4	Bottom	27.94	1.59	2.20	.72	17.57
	Middle	22.18	2.07	2.17	.95	10.72
	Top	18.60	2.15	2.11	1.02	8.65
5	Bottom	27.69	1.63	2.46	.66	16.98
	Middle	24.45	2.09	2.29	.91	11.68
	Top	14.18	2.35	2.59	.91	6.03
6	Bottom	28.42	1.71	2.68	.64	16.62
	Middle	22.63	1.86	2.44	.76	12.17
	Top	13.35	2.32	2.71	.85	5.75
7	Bottom	24.93	1.77	3.18	.56	14.08
	Middle	20.89	1.98	2.81	.70	10.55
	Top	18.62	2.21	2.78	.79	8.44
8	Bottom	22.40	1.80	3.24	.56	12.44
	Middle	25.29	1.85	2.72	.68	13.68
	Top	18.19	2.20	2.74	.80	8.27

1939, BEST QUALITY

1	Bottom					
	Middle	14.00	2.12	0.73	2.91	6.61
	Top	6.54	2.74	1.29	2.12	2.39
2	Bottom	14.15	2.10	1.39	1.51	6.74
	Middle	17.21	2.05	1.46	1.40	8.40
	Top	12.46	2.20	1.51	1.46	5.66
3	Bottom	12.08	2.06	1.82	1.13	5.87
	Middle	17.75	1.84	1.75	1.05	9.65
	Top	9.69	2.31	2.15	1.07	4.19
4	Bottom	13.70	2.21	2.91	.76	6.20
	Middle	22.58	1.93	2.43	.79	11.71
	Top	11.02	2.27	2.57	.88	4.86
5	Bottom	12.24	2.05	3.70	.55	5.98
	Middle	20.09	1.91	3.05	.63	10.51
	Top	8.93	2.36	3.48	.68	3.78
6	Bottom	13.21	2.09	3.82	.55	6.33
	Middle	21.50	1.89	2.99	.63	11.37
	Top	13.60	2.21	2.65	.83	6.15
7	Bottom	14.70	1.95	3.80	.51	7.55
	Middle	20.62	1.92	3.50	.55	10.73
	Top	10.17	2.33	3.38	.69	4.36
8	Bottom	16.85	1.88	4.08	.46	8.96
	Middle	20.97	1.86	3.44	.54	11.30
	Top	9.59	2.21	3.54	.62	4.34

TABLE 3.—*Effect of various fertilizer treatments on certain constituents of the bottom, middle, and top leaves of best-quality bright leaf cigarette tobacco produced on the experimental plots in 1938, and of best and scrap quality in 1939*—Con.

1939, SCRAP QUALITY

Plot No.[1]	Location of leaves	Reducing materials calculated as glucose	Nitrogen	Potassium	Nitrogen/potassium ratio	Glucose/nitrogen ratio
		Percent	*Percent*	*Percent*		
1	Bottom	6.70	2.37	0.61	3.88	2.82
	Middle	12.48	2.13	1.09	1.96	5.86
	Top	3.70	3.12	1.32	2.36	1.19
2	Bottom	7.57	2.27	1.27	1.79	3.33
	Middle	17.22	1.93	1.35	1.43	8.93
	Top	9.12	2.44	1.61	1.52	3.74
3	Bottom	5.54	2.28	1.97	1.16	2.43
	Middle	14.39	1.92	1.89	1.02	7.50
	Top	7.49	2.54	2.44	1.04	2.95
4	Bottom	5.81	2.39	3.08	.78	2.43
	Middle	21.05	1.72	2.44	.71	12.25
	Top	9.86	2.23	2.55	.88	4.42
5	Bottom	7.83	2.16	3.33	.65	3.62
	Middle	16.31	1.98	3.17	.62	8.25
	Top	7.81	2.24	3.44	.65	3.49
6	Bottom	5.07	2.42	4.30	.56	2.09
	Middle	18.05	1.90	3.30	.58	9.51
	Top	9.46	2.41	3.22	.75	3.92
7	Bottom	7.72	2.34	4.53	.52	3.30
	Middle	16.74	1.96	3.34	.59	8.55
	Top	8.35	2.39	3.50	.68	3.49
8	Bottom	8.03	2.23	4.17	.54	3.60
	Middle	17.04	1.72	3.58	.48	9.92
	Top	7.08	2.36	3.48	.68	3.00

[1] See table 1 for formulas and quantities of fertilizers used on these plots.

DISCUSSION OF RESULTS

The results on the intake and accumulation of potassium as indicated in table 3 are somewhat in agreement with the observations of Anderson and coworkers (*1*), who stated that where the supply of potash in the soil was quite low, this element was found in higher concentrations in the upper rather than in the lower leaves. On the other hand, where the supply was ample, there was a higher accumulation in the lower leaves.

That the distribution of potassium in the plant is influenced in a measure by the quantities available is indicated by table 3. In plots 1 and 2 the low level of available potassium appeared insufficient to meet the demands of the growing plant. Under these conditions a larger accumulation of this element should be expected in the upper portions. However, where ample or rather excessive quantities of potassium were available to the plants, it appeared to be absorbed throughout the growth period, resulting in a marked accumulation of this element in the lower plant parts.

The incorporation of relatively large quantities of potassium in the fertilizer mixtures, improperly balanced with other necessary mineral nutrients, does not appear to be advisable, since the market value of the tobacco produced on plots so treated was found to be quite low. The conditions under which these plants were grown, however, indicate that quantities approximating 100 pounds per acre may be desirable.

While it is not advisable to produce bright leaf tobacco containing large quantities of nitrogen, this element must be given due consideration as supplying certain properties to the leaves which are extremely

desirable from the standpoint of both yield and quality. From the results obtained, the writers are inclined to believe that the ratio of nitrogen to potassium should approximate 1:1 in the leaves. (See figs. 1 and 2.) It is apparent that the plots receiving minimum quantities of potassium had a wider ratio, while those receiving excessive quantities had a narrower ratio than this value. Leaves showing a ratio of approximately 1:1 were found on the middle leaves of the plants grown on plots 3, 4, and 5 during the 1938 season.

It would appear that there is some correlation between the nitrogen/potassium ratio of the leaves and the fertilizer treatment of the plants. While the 1938 and 1939 crops were produced under different weather conditions, the figures for the nitrogen/potassium ratio were of somewhat the same order.

Samples of the 1938 crop showed an increase in the concentration of nitrogen from the lower to the upper leaves. However, there appeared to be a slight variation where the potassium supply was more or less ample. On the other hand, the variation was quite pronounced for plot 1 where no potassium was applied. This was likewise noted for samples taken from this plot in 1939, while the remaining plots showed approximately the same concentration of nitrogen in the bottom and top leaves, being somewhat lower in the middle leaves.

It has been stated on numerous occasions that normal carbohydrate formation is closely associated with the presence of ample quantities of potassium (*9*, *8*, *5*). While the quantity of available soil potassium in plot 1 may have been sufficient to meet the needs of the plants during their early period of growth, it apparently was not sufficient to meet their demands during the latter stages of growth, as may be noted in the very low concentration of carbohydrates in the mature plants.

The results presented for the 1938 crop in table 3 are of particular interest. The samples from practically all plots showed a larger quantity of reducing materials in the lower than in the higher leaves. For those plots receiving sufficient potash this difference was less pronounced.

It is quite apparent that the reducing materials of the leaf are more or less associated with its quality. A comparison of the composition of the two grades of tobacco obtained from the 1939 crop, as given in table 3, is striking. In all cases, the reducing materials of the bottom and top leaves of the scrap quality was low, while that of the middle leaves was somewhat higher. It may be noted that while the bottom leaves of samples from plot 4 had approximately the same nitrogen/potassium ratio, the reducing materials of the scrap quality were less than half that of the best quality tobacco.

Harvesting of bright leaf tobacco is accomplished by the removal of two or three leaves at a time as they become "ripe," starting at the bottom and progressing upward. This procedure is called "priming" and in a normal season five or more primings are taken from each plant. It is obvious, therefore, that weather conditions prior to a priming may influence the composition of the leaves. The moisture content of the soil has a great deal to do with the absorption of mineral nutrients by plants and in this connection it should be remembered that above-normal rainfall was experienced in 1938 while rainfall was below normal in 1939.

SUMMARY AND CONCLUSIONS

Samples of bright leaf cigarette tobacco were obtained from each of eight experimental plots where potash was the only variable in the individual fertilizer treatments, ranging from none to 300 pounds per acre. Samples were taken from both the 1938 and 1939 crops and divided into three groups according to their position on the stalk, as bottom, middle, and top leaves. Only the best quality samples of the 1938 crop were analyzed, but in 1939 analyses were made of both scrap and best quality. From the results obtained it may be concluded:

(1) When the quantity of potassium available to the plants is low, a greater concentration of this element occurs in the upper leaves, and the content of reducing materials, including glucose, is quite low in all the leaves.

(2) When the quantity of potash at the disposal of the plants is satisfactory, there is a more or less uniform concentration of this element in the bottom, middle, and top leaves and the production of reducing materials attains a maximum. During the season of 1938 these conditions prevailed where potash was applied at the rate of 90 pounds per acre.

(3) Extremely large applications of potash give rise to an increased absorption of this element, with a larger accumulation in the bottom leaves. Under the conditions of these experiments, such applications of potash, improperly balanced in respect to other nutrient materials, failed to show any further favorable influence on the production of reducing materials than that observed in plants receiving adequate potash fertilization.

(4) Under the conditions of these experiments, a nitrogen/potassium ratio of from 0.8 to 1.1 appears to be highly desirable. Leaves of tobacco plants grown under these varied conditions showed a nitrogen/potassium ratio ranging from 0.5 to 3.9. Neither extreme was found to be associated with good quality.

Desirable nitrogen/potassium ratios were obtained in 1938 from applications of from 60 to 120 pounds of potash, while desirable nitrogen/potassium ratios were obtained in 1939 from 60 to 90 pounds of potash applied on the basis of 1 acre, which may be attributed in large measure to seasonal differences.

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USE OF SODIUM BIFLUORIDE AND SODIUM SILICOFLUORIDE IN THE DISINFECTION OF HIDES [1]

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INTRODUCTION

The importation of hides and skins presents a hazard to the livestock industry of the United States because of the possible introduction of various disease germs or viruses that may adhere to or be incorporated in these materials. Such maladies as foot-and-mouth disease, rinderpest, and anthrax can be spread in this manner. Federal regulations covering the disinfection of hides and skins imported from countries in which such communicable diseases are known to exist, therefore, are rigidly enforced.

Research has been continuous in an effort to find some chemical substance or compound that would be effective in rendering all infected hides and skins safe for importation, without injuring them for tanning purposes. Various chemical substances have been found to be effective germicides or virucides when used with only the etiological agents of various diseases, but in the presence of animal tissue the disinfectants in many instances have been ineffective. This condition is due to the inability of some disinfectants to penetrate the tissues or to the formation of an insoluble substance by the combination of the chemical with the tissue proteins. Another equally important factor in hide disinfection is the effect of the disinfectants on the tanning properties of the hides. Some disinfectants have been found to be effective in destroying the contaminating germs or viruses, but they damaged the hides.

REVIEW OF LITERATURE

Most of the early work on hide disinfection was connected with anthrax. Extensive research was necessary to find methods of disinfection that would destroy both the anthrax bacilli and the spores. The most widely accepted methods were those recommended by Seymour-Jones (*11*) [2] and Schattenfroh (*10*). Work was done by Ponder (*9*), Smyth (*12*), Tilley (*13*), and O'Flaherty and Doherty (*7*) to determine the efficiency of these methods.

The prevention of foot-and-mouth disease in the United States is extremely important because of the enormous losses to livestock owners and the cost to the State and Federal Governments caused by the drastic methods of eradication. Much work has been done by various investigators toward the control of foot-and-mouth disease. The British Foot-and-Mouth Disease Research Committee of the Ministry of Agriculture and Fisheries, in its first (*2*), second (*3*), third (*4*), fourth (*5*), and fifth (*6*) progress reports, and the United States Foot-and-Mouth Disease Commission (*8*) found that agents which coagulate protein are ineffective against the virus in the presence of exudate or tissue. As a result, various noncoagulating protein chemicals were employed and found to be virucidal when used in the proper dilution.

[1] Received for publication January 18, 1941.

[2] Numbers in parentheses refer to Literature Cited, p. 47.

Among the chemicals mentioned are sodium hydroxide, sodium bisulfate, sodium bisulfite, antiformin, sulfoliquid, and formalin. Results obtained by Trautwein and Reppin (*14*), as well as Winkel (*15*), showed that 1 percent of sodium hydroxide killed the virus readily, and the latter worker used 0.1 to 2 percent of sulfuric acid with equal effectiveness. In the 1928 outbreak of foot-and-mouth disease in Bavaria, Germany, 1 percent of sodium hydroxide in conjunction with 5 percent of calcium hydroxide was successfully used to disinfect premises. Helm and Curtze (*1*) demonstrated that the efficiency of sulfoliquid and caustic soda was greatly increased when applied hot (100° C.).

In the third report of the British Foot-and-Mouth Disease Research Committee (*4*), mention is made of the use of formalin in the disinfection of hides heavily infected with foot-and-mouth-disease virus. In such hides soaked in 1-percent formalin solution for 48 hours, the virus was destroyed but the formalin had a detrimental effect on the hides. In the fourth report of that Committee (*5*), the statement is made that when infected hides were soaked in sodium bisulfate solution (1 to 10,000) for 5 hours or sodium bifluoride (1 to 20,000) for 2 hours, the virus was destroyed and no damage was done to the hides from the standpoint of leather production. No further mention was made of this method of hide treatment in subsequent publications nor were the protocols of the experiment given in that report.

To confirm this work, O'Flaherty and Doherty (*7*) used sodium bisulfate and sodium bifluoride in hides contaminated with the virus of vesicular stomatitis rather than foot-and-mouth disease, in accordance with the policy of the Bureau of Animal Industry of the United States Department of Agriculture not to experiment with the virus of foot-and-mouth disease in the United States. This has been a long-established policy because of danger to the livestock industry of the presence of foot-and-mouth disease virus in the country even for experimental purposes. These authors found that sodium bifluoride, in a solution of 1 to 10,000, destroyed the virus in 24 hours, whereas sodium bisulfate, in a solution of 1 to 400, failed to kill in 24 hours. No undesirable influence on the skin and subsequent leather was noticed by several tanneries that used the sodium bifluoride process of treatment.

PURPOSE AND EXPERIMENTAL PROCEDURE

To test the virucidal action of sodium bifluoride and sodium silicofluoride on hides impregnated with the virus of vesicular stomatitis, experiments were conducted in 1939 at the United States Department of Agriculture Animal Disease Station, Beltsville, Md. Virus of vesicular stomatitis was used because of its similarity to the virus of foot-and-mouth disease. The use of sodium bifluoride was suggested by the report of the British Foot-and-Mouth Disease Research Committee showing its value in destroying the virus of foot-and-mouth disease. Sodium silicofluoride was tested because it had been reported to be the most efficient of the fluorine compounds for curing or preserving hides.[3]

The vesicular virucidal virus was of the New Jersey type and was obtained from artificially infected cattle and horses. The lesions from these animals were dried, sealed in vacuo, and held at 4° C.

[3] Test made at the suggestion of C. E. Senseman, Industrial Farm Products Research Division, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

This dried virus was inoculated on scarified metatarsal pads of guinea pigs. Only freshly harvested virus from guinea pigs with high temperatures at 24 to 28 hours and good lesions at 48 hours after inoculation was used in the experiments. The use of a virus after only a single passage through guinea pigs eliminated as much as possible any changes in virulence or structure of the virus that might occur from continued passage in laboratory animals or live-tissue culture.

The tests included two methods of determining the virucidal action of the disinfectants: Use of (1) infected guinea pig pads placed with calfskin in the various soaks, and (2) calfskin artificially impregnated with the virus and placed in the various soaks. In each method, soak solutions were prepared of sodium bifluoride and sodium silicofluoride. Untreated tap water from a well was used as a control. The tests were repeated twice and identical results were obtained unless otherwise stated. The entire experiment was carried out at room temperature (20° to 24° C.).

In the first method, sodium bifluoride and sodium silicofluoride were dissolved in tap water in dilutions of 1 to 5,000, 1 to 10,000, and 1 to 20,000. The three dilutions for each of the two chemicals were placed in hard rubber vats, which were used instead of glass jars to eliminate any possibility of chemical reaction between the fluorine and the elements in glass. During the soaking periods, the vats were covered to prevent the admittance of light, thus eliminating any possible harmful effect on the virus. Hydrogen-ion determinations were made on each dilution before soaking and after 24 and 48 hours of soaking. Infected metatarsal pads from 6 guinea pigs and 10 pieces weighing 50 gm. each of salt-cured calfskin were placed in 2,500 gm. of each dilution of test solution and held at room temperature for 24- and 48-hour periods. At the end of each holding period, half of the guinea pig pads were removed from each dilution of test solution, washed in 0.85-percent saline solution, and ground in a sterile mortar. Then an 0.85-percent saline solution was added in sufficient quantity to make a 10-percent suspension. These suspensions were shaken thoroughly and then allowed to settle. The supernatant fluid from each suspension was inoculated on scarified metatarsal pads of guinea pigs; all pads were covered with sterile gauze for 24 hours. Temperatures were taken on the guinea pigs at 24 to 28 hours after inoculation, and the metatarsal pads were examined for lesions after 48 hours. These guinea pigs were saved for 13 to 21 days and then reinoculated with fresh virus to determine whether any immunity had been acquired as well as to eliminate the possibility of false clinical manifestations that may have been caused by the test solutions. In addition to the control experiments involving the placing of affected guinea pig pads in tap water, control experiments were also carried out with normal guinea pigs to determine the virulence of the virus used in all the soak solutions as well as that used to test the animals for immunity.

In the second method, conditions were the same as in the first except that 1 gm. of infected pads from guinea pigs was ground in a mortar with 5 cc. of 0.85-percent saline solution, and 0.25 cc. of this suspension of virus was then injected intradermically into circular pieces of calfskin, each 1 inch in diameter and weighing approximately 1 gm. Six inoculated pieces were placed between 50-gm. pieces of calfskin in each of the dilutions of sodium bifluoride and sodium sili-

cofluoride. The proportion of the 50-gm. pieces of skin to soak solution was 1 to 5 by weight as in the first method. Half of the impregnated circular pieces of calfskin were removed from the test solutions at 24 hours and the remaining half at 48 hours. In each instance they were washed in 0.85-percent saline solution, cut into very small pieces with sterile scissors, placed in a mortar, and ground to a pulpy consistency. This material was inoculated on guinea pig pads, the remainder of the procedure being the same as described in the first method. The purpose of this type of experiment was to determine the penetrating ability of the soak solution and to duplicate naturally infected skins as nearly as possible.

RESULTS

The results obtained by the first method are shown in table 1. The sodium bifluoride, in the three dilutions used, destroyed the virus of vesicular stomatitis in the guinea pig pads after 24 hours of soaking. Sodium silicofluoride, in dilutions of 1 to 10,000 and 1 to 5,000, destroyed the virus in 24 hours. The 1 to 20,000 dilution killed the virus only after 48 hours of soaking. The virus was still viable after 48 hours of soaking in tap water at room temperature. Results with the normal control guinea pigs demonstrated the virulence of the virus used in all the soak solutions as well as that used to test guinea pigs for immunity.

TABLE 1.—*Effect of sodium bifluoride and sodium silicofluoride on virus of vesicular stomatitis in guinea pig pads, in the presence of salt-cured calfskins, 1939*

Disinfectant				Guinea pig data[1] on—						
				Test of virulence				Test of immunity[2]		
Kind	Dilution	Length of soak	Guinea pig No.	Date of inoculation	Temperature at 24 hours	Lesions, at 48 hours, on—		Temperature at 24 hours	Lesions, at 48 hours, on—	
						Left pad	Right pad		Left pad	Right pad
		Hours			°F.			°F.		
Sodium bifluoride[3]	1- 5,000	24	664	Oct. 10	101.8	o	o	104.6	d	d
	1-10,000		670		101.7	o	o	105.4	d	d
	1-20,000		667		103.2	o	o	105.0	d	d
	1- 5,000	48	825	Oct. 11	103.2	o	o	105.4	c	d
	1-10,000		816		101.6	o	o	105.6	d	d
	1-20,000		818		102.2	o	o	105.9	d	d
Sodium silicofluoride[3]	1- 5,000	24	663	Oct. 10	102.5	o	o	105.6	d	d
	1-10,000		699		101.8	o	o	105.3	d	d
	1-20,000		674		102.9	a	o	104.6	b	d
	1- 5,000	48	822	Oct. 11	102.0	o	o	105.3	d	d
	1-10,000		801		102.4	o	o	105.1	d	d
	1-20,000		815		103.0	o	o	106.1	d	d
Tap water (controls)[4]		24	1078	Oct. 10	105.2	d	d	101.6	o	o
			1087		105.1	d	d	102.4	o	o
			1092		105.0	d	d	102.1	o	o
			1098		105.8	d	d	101.5	o	o
		48	1084	Oct. 11	106.0	d	d	103.0	o	o
			1089		106.0	d	d	102.1	o	o
			1090		105.8	d	d	102.5	o	o
			1093		106.4	d	d	102.6	o	o
None (normal controls)			1	Oct. 9	105.4	d	d			
			2		105.1	d	d			
			3		105.6	d	d			
			4		106.0	d	d			
			5					106.2	d	d
			6					105.0	d	d
			7					105.2	d	d
			8					105.7	d	d

[1] Key: o, none; a, slight (swelling and tenderness); b, moderate (swelling and tenderness plus a few small vesicles along the hair line); c, good (pad loosened, tenderness plus incomplete vesicle formation around and under entire pad); d, severe (pad loosened, complete vesicle formation under and around pad and sloughing).

[2] Fresh virus of vesicular stomatitis from guinea pig pads was used; date of inoculation in all instances, Oct. 28,1939.

[3] 12 affected guinea pig pads used in each dilution of the soak solution.

[4] 12 affected guinea pig pads used.

The results obtained by the second method are shown in table 2. With sodium bifluoride, in dilutions of 1 to 10,000 and 1 to 5,000, the virus was destroyed after 24 hours of soaking. In the 1 to 20,000 dilution, the virus was destroyed in 50 percent of the cases in 24 hours, but the destruction of virus was complete only after 48 hours of soaking.

With sodium silicofluoride, 1 to 10,000 and lower dilutions destroyed the virus in 24 hours, whereas the 1 to 20,000 dilution completely destroyed the virus in only 50 percent of the cases after 48 hours of soaking.

TABLE 2.—*Effect of sodium bifluoride and sodium silicofluoride on virus of vesicular stomatitis inoculated into salt-cured calfskins, 1939*

Disinfectant				Guinea pig data[1] on—						
				Test of virulence				Test of immunity[5]		
Kind	Dilution	Length of soak	Guinea pig No	Date of inoculation	Temperature at 24 hours	Lesions, at 48 hours, on—		Temperature at 24 hours	Lesions, at 48 hours, on—	
						Left pad	Right pad		Left pad	Right pad
		Hours			°*F.*			°*F.*		
Sodium bifluoride[3]	1– 5,000	24	975	Oct. 18	102.0	o	o	104.4	d	d
	1– 5,000		755		102.7	o	o	105.1	d	d
	1–10,000		974		102.9	o	o	105.4	d	d
	1–10,000		874		103.0	o	o	104.8	d	d
	1–20,000		858		103.4	o	o	105.0	d	d
	1–20,000		964		103.6	b	a	103.0	a	a
	1– 5,000	48	948	Oct 19	102.8	o	o	104 4	d	d
	1– 5,000		932		102.7	o	o	105.5	c	c
	1–10,000		968		102.0	o	o	105.3	d	d
	1–10,000		788		102.0	o	o	104.8	d	d
	1–20,000		970		102.0	o	o	105.1	d	d
	1–20,000		928		103.0	o	o	105.0	d	d
Sodium silicofluoride[3]	1– 5,000	24	865	Oct. 18	102.4	o	o	104.7	d	d
	1– 5,000		867		103.1	o	o	104.4	d	d
	1–10,000		871		103.0	o	o	103.8	c	c
	1–10,000		866		102.6	o	o	104.6	d	d
	1–20,000		851		102.8	o	a	104.6	d	c
	1–20,000		870		103.3	a	o	102.6	b	b
	1– 5,000	48	936	Oct. 19	101.7	o	o	104.5	d	d
	1– 5,000		937		101.9	o	o	105.8	d	d
	1–10,000		942		103.0	o	o	105.3	d	d
	1–10,000		945		102.5	o	o	104.8	d	d
	1–20,000		927		103.0	o	a	103.6	b	a
	1–20,000		949		102.8	o	o	104.6	d	d
Tap water (controls)[4]		24	1082	Oct. 18	105.2	d	d	101.8	o	o
			943		105.5	d	d	102.4	o	o
			1097		104.8	d	d	102.2	o	o
			1069		106.2	d	d	101.4	o	o
		48	1001	Oct. 19	104.5	d	d	102.0	o	o
			1071		105.6	d	d	101.8	o	o
			1076		105.0	d	d	102.6	o	o
			1080		104.8	d	d	102.2	o	o
None (normal controls)			9	Oct. 17	105 2	d	d			
			10		105.0	d	d			
			11		105.6	d	d			
			12		105.4	d	d			
			956					105.1	d	d
			910					104.9	d	d
			955					105.4	d	d
			777					104.6	d	d

[1] Key: o, none; a, slight (swelling and tenderness); b, moderate (swelling and tenderness plus a few small vesicles along the hair line); c, good (pad loosened, tenderness plus incomplete vesicle formation around and under entire pad); d, severe (pad loosened, complete vesicle formation under and around pad and sloughing).

[2] Fresh virus of vesicular stomatitis from guinea pig pads was used; date of inoculation in all instances, Oct. 31, 1939.

[3] 6 1-gm. pieces of calfskin, each injected with 0.25 cc. of affected guinea-pig-pad emulsion, used in each dilution of soak solution.

[4] 6 1-gm. pieces of calfskin, each injected with 0.25 cc. of affected guinea-pig-pad emulsion, used.

The virus was still viable after 48 hours of soaking in tap water. Similar results were obtained with the control guinea pigs as in the first method.

The hydrogen-ion concentration of tap water and the various solutions is shown in table 3. All the soaks tended to be less acid with increase in length of time, but none of the solutions reached alkalinity. However, the virucidal action of these solutions was not comparable with the degree of acidity as sodium silicofluoride in the comparative dilutions had in almost every instance a higher acidity than sodium bifluoride, whereas sodium bifluoride, in the virulence tests, was the more virucidal.

TABLE 3.—*Average hydrogen-ion concentration of the soak solutions at various periods in all experiments*

Soak solution	pH value—		
	Before soaking	After 24 hours	After 48 hours
Sodium bifluoride:			
1–5,000	3.7	4.7	5.4
1–10,000	3.75	5.6	5.9
1–20,000	3.8	6.0	6.2
Sodium silicofluoride:			
1–5,000	3.5	4.6	5.0
1–10,000	3.55	5.2	5.7
1–20,000	3.6	5.7	6.2
Tap water	6.6	6.85	7.2

DISCUSSION

Results obtained from the studies indicate that both sodium bifluoride and sodium silicofluoride are virucidal to the virus of vesicular stomatitis. Sodium bifluoride, in a dilution of 1 to 20,000, was slightly more effective than sodium silicofluoride under like conditions.

A longer time was required for the higher dilutions of the disinfectants to kill the virus injected intradermically in hides than to kill the virus in the guinea pig pads. This fact was due, no doubt, to the extra time required by the disinfectants to penetrate the skin before coming in contact with the virus. After the skins had been in the various soak solutions for 24 to 48 hours, a greater thickness was noted in the skins soaked in the two disinfectant solutions than in those soaked in tap water.

It appears that the sodium chloride in the cured skins had little influence on the effectiveness of sodium bifluoride or sodium silicofluoride, as its presence on the skins in tap water failed to affect the virulence of the virus.

The importance of sodium bifluoride and sodium silicofluoride for use in hide and skin disinfection depends on their effectiveness against foot-and-mouth disease virus. The present experiments showed that these disinfectants destroyed vesicular stomatitis virus after 24 hours of soaking in dilutions of 1 to 10,000. The British Foot-and-Mouth Disease Research Committee in its fourth report (*5*) states that a dilution of 1 to 20,000 of sodium bifluoride killed foot-and-mouth disease virus in 2 hours, and O'Flaherty and Doherty (*7*) found that

a dilution of 1 to 10,000 of sodium bifluoride destroyed vesicular stomatitis virus after 24 hours.

From these data it may be assumed that the use of either sodium bifluoride or sodium silicofluoride, in a solution of 1 to 10,000 for 24 hours at room temperature, would be effective in the disinfection of hides or skins infected with foot-and-mouth disease when the ratio of hide or skin to soak solution is 1 to 5 by weight.

SUMMARY AND CONCLUSION

The efficiency of sodium bifluoride and sodium silicofluoride as hide disinfectants was studied, the virus of vesicular stomatitis being used as the contaminant. This work was carried on at the United States Department of Agriculture Animal Disease Station, Beltsville, Md., in 1939.

Two methods of approach were used: (1) Infected guinea-pig pads soaked for 24 and 48 hours in solutions of 1 to 5,000, 1 to 10,000, and 1 to 20,000 of the two fluorine compounds, in the presence of salt-cured calfskins; (2) sections of salt-cured calfskin injected intradermically with vesicular stomatitis virus in the aforementioned soak solutions. In both types of experiments, tap water was used as a control. The proportion of salt-cured skin to the quantity of soak solution in all experiments was 1 to 5 by weight. All experiments were conducted at room temperature.

The results were as follows:

In the first experiment, sodium bifluoride killed the virus in guinea-pig pads in all three dilutions in 24 hours. Sodium silicofluoride killed the virus in dilutions of 1 to 5,000 and 1 to 10,000 in 24 hours and in all dilutions in 48 hours.

In the second experiment, sodium bifluoride killed the virus in artificially inoculated calfskins in dilutions of 1 to 5,000 and 1 to 10,000 in 24 hours and in all dilutions in 48 hours. Sodium silicofluoride killed the virus in dilutions of 1 to 5,000 and 1 to 10,000 in 24 hours but was not completely effective in the dilution of 1 to 20,000 in 48 hours.

Tap water did not affect the virus in either guinea-pig pads or calfskins after 24 or 48 hours of soaking.

The hydrogen-ion concentration of the various soak solutions was determined at the beginning and after 24 and 48 hours of soaking. Sodium silicofluoride, at equal dilutions, had a higher hydrogen-ion concentration than sodium bifluoride, both decreasing with length of time. However, a high hydrogen-ion concentration does not necessarily indicate greater virucidal powers. The hydrogen-ion concentration of tap water also decreased with length of time.

By analogy with similar research by the British Foot-and-Mouth Disease Research Committee, it is a logical assumption that sodium bifluoride and sodium silicofluoride are also effective in destroying the virus of foot-and-mouth disease.

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THE EFFECT OF FERTILIZATION AND CULTURAL PRACTICES ON THE OIL AND AMMONIA CONTENT OF COTTONSEED GROWN ON YAZOO-MISSISSIPPI DELTA SOILS[1]

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INTRODUCTION

The cotton-oil mills of the Yazoo-Mississippi Delta usually pay a premium on cottonseed grown in that area. Since this is one of the world's greatest cotton producing areas, it becomes a question of considerable importance to both the producer and processor to know what factors are responsible for this quality product so that greater use may be made of this apparent natural advantage in the highly competitive field of cotton production.

The market value of cottonseed is determined by the percentages of oil, meal, hulls, and linters which the seeds contain. Pound for pound, the oil is the most valuable constituent of the seed, but since the percentage of meal is roughly three times that of the oil, the meal represents the higher total money value per acre. Garner et al.[3] have shown that cottonseed produced on different types of soil may differ somewhat in its content of oil and ammonia. Certain oil-mill records[4] indicate that cottonseed produced on the alluvial soils of the Yazoo-Mississippi Delta, which are among the most productive soils in the Cotton Belt, may contain a higher percentage of both oil and ammonia than cottonseed produced in the upland or less fertile parts of Mississippi.

Cultural practices in cotton production are not uniform, even in the same section, and any relationship that might be established between soil fertility and the composition of cottonseed would not have its widest application without a knowledge of the effect, if any, produced by different cultural practices. To determine to what extent these variations in cultural methods might affect the oil and ammonia content of the cottonseed, a study of the effect of the cultural pactices most commonly followed in the Yazoo-Mississippi Delta was included in this investigation.

METHODS AND MATERIALS

Samples of cottonseed were collected from $\frac{1}{20}$-acre field plots, replicated five to nine times, at the Delta Branch Experiment Station, Stoneville, Miss., and analyzed for oil, ammonia, and moisture content in accordance with the methods of the National Cottonseed Products Association. The data are reported on a 10-percent moisture basis.

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[2] The valuable assistance of Dr. Rowland Cowart of this station in the preparation of the manuscript is gratefully acknowledged.

[3] GARNER, W. W., ALLARD, H. A., and FOUBERT, C. L. OIL CONTENT OF SEEDS AS AFFECTED BY THE NUTRITION OF THE PLANT. Jour. Agr. Res. 3: 227-249. 1914.

[4] Unpublished records of the Tallahatchie Oil Mill, Webb, Miss.

The plots from which the samples were taken had received annually uniform treatments for a period of 10 years prior to the first sampling. The sampling was made annually from 1933 to 1937, inclusive.

The fertility treatments or cultural practices may be grouped as follows: (1) Commercial fertilizers, which supplied nitrogen from different sources and at different rates, phosphorus from superphosphate, and potassium from sulfate of potash at the rate of 45 pounds P_2O_5 and 37½ pounds K_2O per acre, respectively; (2) green manures, which included hairy vetch, Austrian winter peas, sweetclover, burclover, and rye; and (3) cultural practices, which included (a) Variations in methods of seedbed preparation consisting of no plowing, bedding in fall, bedding in spring, and bedding in fall and rebedding in spring, all followed by uniform cultivation; (b) uniform seedbed preparation, followed by weekly cultivations for each depth, which included hoeing only, harrowing, cultivating 3, 6, and 6 inches deep followed by 3 inches deep; and (c) variations in spacing of plants within the hills, the number of plants per hill ranging from two to seven with a uniform distance of 16 by 40 inches between hills.

EXPERIMENTAL RESULTS

COMMERCIAL FERTILIZERS

NITROGEN

The data in table 1 show the effect which 30 pounds of nitrogen per acre applied in the form of various commercial nitrogenous fertilizers had on the oil and ammonia content of cottonseed grown on Sarpy loam soil. There seems to have been no effect on the oil content of the cottonseed regardless of the source of nitrogen, but the ammonia content was increased considerably by nitrogen from all of the different sources, though least by that from organic sources.

TABLE 1.—*Effect of nitrogen from different sources on the yield and on the oil and ammonia content* [1] *of cottonseed grown on Sarpy loam*

Source of nitrogen (30 pounds per acre)	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Percent*	*Percent*
No nitrogen	616	20.88±0.10	3.41±0.03
Sodium nitrate	980	20.19± .25	3.71± .07
Ammonium nitrate	935	20.37± .20	3.66± .09
Ammonium sulfate	916	21.05± .18	3.66± .07
Cyanamid	914	20.36± .19	3.62± .06
Cottonseed meal	975	20.51± .15	3.53± .13

[1] Standard error is used to indicate the degree of significance.

Table 2 gives the data on the performance of nitrogen from two sources, sodium nitrate and cyanamid, applied at rates ranging from 7½ to 45 pounds of nitrogen per acre at 7½-pound intervals. Nitrate of soda is immediately soluble, but cyanamid has to undergo certain chemical changes in the soil before the nitrogen is available to the plants. However, the general trend in the behavior of the two types of nitrogen carriers at the different rates of application was essentially the same. At the higher rates the oil percentages were lower for both sources, but the yield of oil per acre was greater since with the higher rates of fertilization a greater quantity of cottonseed was

produced per acre. The percentage of ammonia in the cottonseed increased with the higher rates of application of nitrogen. Since the higher rates of nitrogen produced more pounds of cottonseed per acre, the higher rates likewise increased the total amount of ammonia to a considerable extent. While some of the increase in percentage of ammonia and the decrease in percentage of oil at the lower rates of nitrogen are not statistically significant, a trend is exhibited in both cases.

TABLE 2.—*Effect of applying at different rates nitrogen derived from sodium nitrate and cyanamid on the yield and on the oil and ammonia content* [1] *of cottonseed grown on Sarpy loam*

Nitrogen applied per acre (pounds)	Results with sodium nitrate			Results with cyanamid		
	Yield of cottonseed per acre	Oil content	Ammonia content	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Percent*	*Percent*	*Pounds*	*Percent*	*Percent*
0	579	20.87±0.15	3.44±0.14	1,030	19.48±0.26	3.97±0.06
7½	656	20.83± .10	3.43± .03	1,052	19.47± .15	4.00± .03
15	724	20.58± .10	3.53± .03	1,066	19.54± .14	3.95± .06
22½	804	20.58± .13	3.63± .05	1,066	19.44± .12	4.06± .02
30	887	20.12± .21	3.71± .04	1,112	19.29± .13	4.07± .02
37½	946	19.89± .28	3.78± .05	1,113	19.13± .12	4.24± .08
45	987	19.81± .33	3.83± .05	1,150	19.01± .12	4.11± .06

[1] See footnote 1, table 1.

TABLE 3.—*Effect of phosphorus and potassium, when nitrogen was supplied by sodium nitrate and cyanamid, on the yield and on the oil and ammonia content* [1] *of cottonseed grown on Sarpy loam*

Treatment	Results with sodium nitrate (30 pounds of nitrogen per acre)			Results with cyanamid (30 pounds of nitrogen per acre)		
	Yield of cottonseed per acre	Oil content	Ammonia content	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Percent*	*Percent*	*Pounds*	*Percent*	*Percent*
None	1,028	19.47±0.30	3.92±0.05	963	19.49±0.18	4.17±0.05
Phosphorus [2]	1,092	19.70± .14	3.96± .06	935	19.20± .13	4.21± .02
Phosphorus and potassium [3]	1,059	20.10± .14	3.94± .07	983	19.60± .23	4.13± .03
Potassium [3]	1,059	19.76± .13	3.92± .04	983	19.33± .21	4.09± .07

[1] See footnote 1, table 1. [2] 45 pounds per acre of P_2O_5. [3] 37½ pounds per acre of K_2O.

PHOSPHORUS AND POTASSIUM

Table 3 shows the results from adding phosphorus and potassium with applications of sodium nitrate and cyanamid to make mixed fertilizers carrying 45 pounds of P_2O_5, 37½ pounds of K_2O, and 30 pounds of nitrogen per acre. The ammonia content of the seed was not appreciably affected in any case. It will be observed also that when phosphorus or potassium was used either alone or in combination, the effect on the oil content of the cottonseed was not statistically significant. These results are in agreement with those of Garner, Allard, and Foubert,[5] who reported that nitrogen affects the oil and

[5] See footnote 3.

ammonia content of cottonseed but that phosphorus and potassium do not. They are not, however, in full agreement with the results of O'Kelly, Hull, and Gieger,[6] who found that the fertilization of cotton with potash raised the oil content but lowered the protein content of the seed. This difference in results may be due in part at least to a difference in the natural supply of potash in the soils used in the two investigations. The work of O'Kelly et al. was carried out on soils so deficient in phosphorus and potassium that cotton responded well to phosphate and potash fertilization, whereas the present work was conducted on a soil that ordinarily has not given economical increases in cotton yield from either phosphate or potash fertilization. It is known that a soil extremely deficient in an element will give a greater response in yield to applications of that element than will soils only moderately deficient in it.

GREEN MANURES

The availability of the nutrients supplied in green manures and the effect of organic matter on soil moisture are two factors that may cause the plant nutrients supplied through commercial fertilizers and green manures to affect the oil and ammonia content of the cottonseed differently. To reduce this source of variability, a number of green manure crops were included in the test, namely, hairy vetch, Austrian winter peas, sweetclover, bur-clover, and rye. Table 4 gives the data on the effect of turning under green manures on the oil and ammonia content of cottonseed. Since some of the crops made more growth than others, different quantities of nitrogen were turned under in the different legumes, a condition which is beyond control in field experimentation. The results with the legumes are similar to those obtained with the commercial sources of nitrogen, that is, the oil percentage was decreased and the ammonia percentage increased by the addition of nitrogenous manure. The results with rye, which is a nonleguminous crop, differed from those of the nitrogenous fertilizers but were very similar to those obtained on the no-treatment plot.

CULTURAL PRACTICES

Since the method of preparing the seedbed, the frequency and depth of cultivation, and the number of plants grown per hill, are practices that vary widely, not only between different cotton-growing regions, but within a single community, an attempt was made to include a wide range of these practices in the investigations. The effect on yield and on oil and ammonia content of the cottonseed resulting from these variations in cultural practices are shown in tables 5, 6, and 7. It will be observed that none of the variations in seedbed preparation, cultivation, or spacing seem to have had any marked effect on the oil or ammonia content of the seed. Any outstanding advantage of one of these practices over another in the growing of cotton would have to be determined on the basis of quantity production and economics rather than on the oil and ammonia content of the seed produced.

[6] O'KELLY, J. F., HULL, W. W., and GIEGER, M. EFFECTS OF VARYING AMOUNTS OF POTASH ON OIL AND PROTEIN AND ON THE WEIGHT AND PERCENTAGE OF COTTONSEED. Miss. Agr. Expt. Sta. Tech. Bul. 20, 8 pp., illus. 1933.

TABLE 4.—*Effect of green manures on the yield and on the oil and ammonia content* [1] *of cottonseed grown on Sarpy loam*

Green manure used	Nitrogen supplied	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Pounds*	*Percent*	*Percent*
None		712	21.20±0.17	3.43±0.07
Austrian winter peas	75	1,071	19.64± .16	3.67± .07
Hairy vetch	85	1,165	19.35± .16	3.68± .06
Sweetclover	58	940	19.89± .22	3.48± .07
Burclover	114	931	19.55± .30	3.59± .09
Rye		603	20.43± .22	3.23± .07

[1] See footnote 1, table 1.

TABLE 5.—*Effect of varying the method of seedbed preparation on the yield and on the oil and ammonia content* [1] *of cottonseed grown on Sarpy loam and on Sharky clay soil*

Method of seedbed preparation	Results on Sarpy loam soil			Results on Sharky clay soil		
	Yield of cottonseed per acre	Oil content	Ammonia content	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Percent*	*Percent*	*Pounds*	*Percent*	*Percent*
None, stalks pulled	531	19.11±0.37	3.85±0.05	571	19.33±0.23	3.91±0.05
Bedded in fall	768	19.53± .33	3.71± .04	570	19.34± .27	3.89± .06
Bedded in spring	808	19.09± .18	3.83± .05	697	19.51± .16	3.95± .05
Bedded in fall and rebedded in spring	874	19.03± .15	3.82± .04	717	19.47± .15	3.86± .05

[1] See footnote 1, table 1.

TABLE 6.—*Effect of varying the method, frequency, and depth of cultivation on the yield and on the oil and ammonia content* [1] *of cottonseed grown on Sarpy loam*

Kind of cultivation	Interval of cultivation	Yield of cottonseed per acre	Oil content	Ammonia content
		Pounds	*Percent*	*Percent*
None, hoe only	Weekly	923	19.43±0.27	3.92±0.05
Harrow, 1 inch deep or less	do	944	18.87± .21	3.67± .06
Plow, 3 inches deep	Semiweekly	1,050	18.98± .32	3.94± .06
Do	Weekly	1,039	18.78± .16	3.98± .05
Plow, 6 inches deep	do	919	18.90± .20	3.96± .03
Plow, 6 inches deep (3 times at weekly intervals) and plow 3 inches deep afterwards	do	1,003	19.28± .12	3.92± .06

[1] See footnote 1, table 1.

TABLE 7.—*Effect of number of plants per hill on yield and on oil and ammonia content* [1] *of cottonseed grown on Sarpy loam*

[Hills spaced 16 by 40 inches]

Plants per hill (number)	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Percent*	*Percent*
2	696	19.28±0.08	3.85±0.09
3	698	19.40± .17	3.83± .05
4	724	19.53± .14	3.86± .06
5	737	19.51± .19	3.85± .05
6	719	19.41± .24	3.92± .04
7	714	19.45± .17	3.98± .09

[1] See footnote 1, table 1.

SUMMARY

Samples of cottonseed were collected annually over a 5-year period from 1933 to 1937, inclusive, on plots of Sarpy loam located at the Delta Branch Experiment Station, Stoneville, Miss., which had received for a 10-year period prior to the first sampling the following treatments: (1) Commercial fertilizers, which included nitrogen, phosphorus, and potash; (2) green manures, which included hairy vetch Austrian winter peas, sweetclover, bur clover, and rye; and (3) a variety of cultural practices, which included (*a*) different methods of seedbed preparation, namely, no plowing, bedding in the fall, bedding in the spring, bedding in the fall and rebedding in the spring; (b) uniform seedbed preparation followed by different methods of cultivation, which included hoeing only, harrowing only, cultivating 3 inches deep, 6 inches deep, 6 inches deep followed by 3 inches deep, and (*c*) variations in number of plants per hill with uniform spacing between hills.

The percentage of oil and ammonia was determined on all samples of cottonseed, with the following results: (1) Nitrogenous fertilizers decreased the percentage of oil in the seed but increased the percentage of ammonia; (2) phosphorus and potassium when used separately gave no increase in oil percentage, but when used together gave a slight increase, although its significance may be questioned; the percentage of ammonia was unaffected in either case; (3) green manures, like commercial fertilizers, increased the percentage of ammonia and decreased the percentage of oil on the basis of their nitrogen content.

The different methods used in preparing the seedbed, cultivating and spacing showed little if any influence on the percentage of oil and ammonia in the cottonseed. The quantity of oil and ammonia produced per acre was influenced somewhat by the different cultural practices, but any advantage of one practice over another is better measured by the quantity of oil and ammonia produced on the basis of acre yield than by the percentage of oil and ammonia in the cottonseed. Nitrogen in whatever form applied increased the ammonia content of the cottonseed.

FURTHER STUDIES OF THE PHOTOPERIODIC BEHAVIOR OF SOME MINTS (LABIATAE)[1]

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INTRODUCTION

The following paper is a partial report of studies of some of the members of the Labiatae begun long ago, the results of which, in part, have been reported in a recent paper.[2]

In the present paper a report is made upon a number of well-known commercial species grown in the United States for their valuable aromatic oils. It seems particularly desirable to learn as much as possible of the ecological requirements of these commercial species, some of which are rather extensively cultivated in certain regions of the United States at the present time.

Since cultivation of some of these mints may be extended and problems of crossing that are being investigated require a timely synchronization in order to secure flowers and pollination of certain species that do not normally have the same flowering season, the following data may be of some interest to those engaged in such work.

MATERIAL[3] AND METHODS

The following species and strains were used in the work herein reported:[3] *Monarda didyma* L., *M. punctata* L., *Mentha arvensis* L., *M. citrata* Ehrh., *M. piperita* L., *M. spicata* L. (*M. viridis* L.), and *M. piperita* var.[4]

Clumps of each of these plants were taken from material growing in the field at Arlington, Va., on May 1, when the plants had just started to produce new growth. On this date good-sized clumps were transplanted to large, galvanized-iron buckets of 14-quart capacity, and the tests were begun May 2, at the Arlington Experiment Farm, Arlington, Va., at approximately lat. 39° N. and with a maximum length of day of 14.9 hours on June 21.

Lengths of day of 10, 12, 12½, 13, 13½, 14, 14½ hours, full day length, and 18 hours were afforded the plants. For all plants, except those receiving 18 hours of illumination daily, natural daylight was used. For photoperiods of 10 to 14½ hours inclusive, the natural daylight period was shortened by means of ventilated lightproof houses into which the plants were run on a rigid schedule each day to obtain the required number of hours of daily exposure. For this purpose large ventilated houses were used, equipped with tracks upon which large movable trucks bearing the containers were mounted.

[1] Received for publication May 2, 1941.

[2] Allard, H. A., and Garner, W. W. Further observations on the response of various species of plants to length of day. U. S. Dept. Agr. Tech. Bul. 727, 64 pp., illus. 1940. (See pp. 39–45.)

[3] Except where otherwise noted, the material reported upon was kindly supplied by the Division of Drug and Related Plants, Bureau of Plant Industry.

[4] This unidentified variety of *M. piperita* was obtained from Vick Chemical Co., Greensboro, N. C.

Before the completion of the experiments, the longer daily light periods were terminated by the natural seasonal shortening of the days. After this time the plants experienced the normal length of day with its daily decrements until the tests were discontinued. On the basis of the periods between actual sunrise and sunset, the 14½-hour day was terminated by the seasonal shortening on July 23; the 14-hour day was terminated on August 8; the 13½-hour day, on August 21; the 13-hour day, on September 3; the 12½-hour day, on September 15; and the 12-hour day, on September 27. However, before and after sunset there is some effective light that may extend the length of day perhaps ½ to ¾ of an hour. If this increase amounted to ¾ of an hour each day, the 14½-hour day would not terminate until August 13, and the 14-, 13½-, 13-, 12½- and 12-hour days would extend to August 25, September 8, September 19, October 1, and October 12, respectively, a period amounting to a little more than 2 weeks longer than when sunrise and sunset alone are considered as delimiting the effective daily light period.

The 18-hour exposures required the use of artificial electric light from sunset, and the duration of this supplemental light was regulated automatically by time clocks and switches, to afford an unbroken period of illumination up to 18 hours each day throughout the season. As the length of day naturally declined after June 21, when the maximum length of day was attained, the period of artificial lighting was increased to compensate for the natural decrements from week to week, just as it was decreased before June 21 as the seasonal day increased.

The artificial supplemental light was supplied by four 200-watt gas-filled tungsten lights fitted with bowl reflectors, one light being placed at each corner of a square metal frame which was 3 feet on the side from center to center of the lights. The lights were so arranged that they could be raised or lowered, and were kept at a distance of 1 foot above the plants. The light intensity of the area beneath this square at a distance of 1 foot amounted to about 300 to 400 foot-candles, as measured by a Weston illumination meter, model 1746, equipped with a Viscor filter designed to measure only visible radiation.

EXPERIMENTAL RESULTS AND DISCUSSION

Great differences were ultimately revealed in the behavior of the various mints tested, some remaining completely vegetative and failing to flower on the shorter lengths of day. There were also marked differences in the production of the prostrate, elongate type of superficial stolons, which serve as very efficient propagating units in the natural extension of the colonies.

In table 1, the characteristic behavior of the various species observed is shown.

The two *Monarda* species tested, *M. didyma* (fig. 1) and *M. punctata* (fig. 2), are native American species, the former being known as Oswego tea or American beebalm, some forms of which are highly prized as showy garden ornamentals. These, unlike the *Mentha* species, do not produce long trailing or prostrate stolons with long internodes and reduced leaves.

TABLE 1.—*Time of flowering and growth behavior of species of Monarda and Mentha in response to different constant daily light periods and full day*

Species and light period (hours)	Date when—		Height—		Stolons		Growth behavior
	Budded	Flowered	At flowering	Sept. 16	Number	Longest	
			Inches	*Inches*		*Inches*	
Monarda didyma:							
10	June 24	July 15	24	25	None		Heads dry, mature Sept. 16.
12	None						Never flowered; reason unknown.
13	June 15	July 5	28	29	None		Heads dry, mature Sept. 16.
14	June 11	July 1	26	25	do		Do.
18	June 1	July 2	28	27½	do		Do.
Full day	June 11	July 5	26	28	do		Do.
Monarda punctata:							
10	June 29	July 23	33	33	None		Do.
12	June 15	July 10	26	25½	do		Do.
13	June 11	June 28	22½	25	do		Do.
14	June 15	July 23	28	28	do		Do.
18	June 14	July 2	29	30	do		Do.
Full day	June 1	July 5	33	33	do		Do.
Mentha arvensis:							
10	July 20	July 29	24	31	Few	34	Still flowering Sept. 16.
12	Aug. 2	Aug. 7	28	34	Very few	34	Still flowering Sept. 16 at tips of branches.
13	July 20	July 29	28	35	Few	19	Still flowering Sept. 16.
14	July 22	do	32	40	Many	34	Still flowering Sept. 16; stolons of large size, vigorous.
18	Aug. 7	Aug. 23	42	46	Few	18	In full flower Sept. 16.
Full day	do	Aug. 27	36	36	Very few	35	Do.
Mentha citrata (bergamot mint):							
10	None	None		23	Many	36	Very vigorous leafy plants; stolons very abundant, long.
12	do	do		25	do	21	Vigorous, leafy plants.
13	Aug. 27	Sept. 23	28½	27	Fewer	29	Moderate development of stolons as compared with 10 and 12 hours.
14	Aug. 23	do	31	30	Rather numerous.	40	A considerable number of stolons.
18	July 29	Sept. 3	34	35	Few	12	Stolons few, short.
Full day	Aug. 14	Sept. 16	31	30	do	30	Just coming into flower Sept. 16.
Mentha piperita (peppermint):							
10	None	None		19	Many	29	Very leafy plants with numerous stolons.
12	do	do		18½	do		Many stolons as in 10-hour test.
13	do	do		21	Fewer	29	Stolons fewer, not so long as in 10- and 12-hour tests.
14	July 13	July 29	31	34	do	23	These plants barely able to flower; stems long, vegetative, 1 alone producing a few flowers; stolons numerous.
18	July 12	do	27	28	Very few	16	Very floriferous and in full flower Sept. 16; very few stolons.
Full day	July 9	do	31	33	Few	17	Past flowering Sept. 16; stolons few.
Mentha spicata (spearmint):							
10	None	None		25	Many	40	Very leafy, vigorous, with many long stolons.
12	July 1	July 29	25½	26	do	34	Past flowering Sept. 16.
13	June 21	July 12	25	29	do	40	Past flowering Sept. 16; many long, much-branched stolons.
14	June 19	July 7	26	29	Fewer	40	Past flowering Sept. 16.
18	June 18	July 8	29	29	Few	9	Past flowering Sept. 16; stolons few, very short.
Full day	June 15	July 10	27	28	do	28	Past flowering Sept. 16; few stolons, and these of moderate length.

TABLE 1.—*Time of flowering and growth behavior of species of Monarda and Mentha in response to different constant daily light periods and full day*—Continued

Species and light period (hours)	Date when—		Height—		Stolons		Growth behavior
	Budded	Flowered	At flowering	Sept. 16	Number	Longest	
			Inches	*Inches*		*Inches*	
Mentha piperita var.[1]:							
10	July 29	Aug. 5	23	33	Many	38	Still in flower Sept. 16; many vigorous stolons.
12	July 20	July 30	27	40	Fewer	18	Still flowering vigorously Sept. 16.
13	July 13	July 23	30	43	Very few	17	Still flowering vigorously Sept. 16; very few stolons.
14	July 13	July 29	30	40	Few	12	A few flowers at the tips of the flower stems.
18	July 10	July 23	32	42	Very few	11	In full flower Sept. 16; 2 short stolons only.
Full day	July 9	do	28	40	do	7	Still in flower Sept. 16.

[1] Originally from Vick Chemical Co., Greensboro, N. C. Near *Mentha piperita*, and perhaps a variety of peppermint (*M. piperita*).

The two species of *Monarda* (table 1) behaved as typical indeterminate or day-neutral plants, since the time of flowering and the increase in height appear to have been little affected by marked changes in length of day. There may have been a tendency to flower somewhat early on the longer light periods, but the tendency was not pronounced.

FIGURE 1.—*Monarda didyma* L. exposed to various day lengths. 10-hour day: Budded June 24; flowered July 15 at 24 inches. 12-hour day: Never budded, not normal. 13-hour day: Budded June 15; flowered July 5 at 28 inches. 14-hour day: Budded June 11; flowered July 1 at 26 inches. 18-hour day: Budded June 1; flowered July 2 at 28 inches. Full day (C): Budded June 11; flowered July 5 at 26 inches. Tests began May 2. Photographed July 25.

Several species of *Mentha* (table 1) show decidedly long-day tendencies. This is indicated by the complete failure of flowering as the days were shortened, in the case of *M. citrata*, *M. piperita*, and *M. spicata*.

In *Mentha arvensis* (fig. 3) and in *M. piperita* var. (fig. 4), a possible hybrid, the long-day relationship is scarcely evident, since flowering continued down to the shortest period of 10 hours of constant daily illumination. Even here, however, it is to be noted that flowering,

in the case of *M. piperita* var., was slightly advanced by the long day of 18 hours. In this species a marked retardation of growth took place with respect to the erect branches of the plant on the shorter day

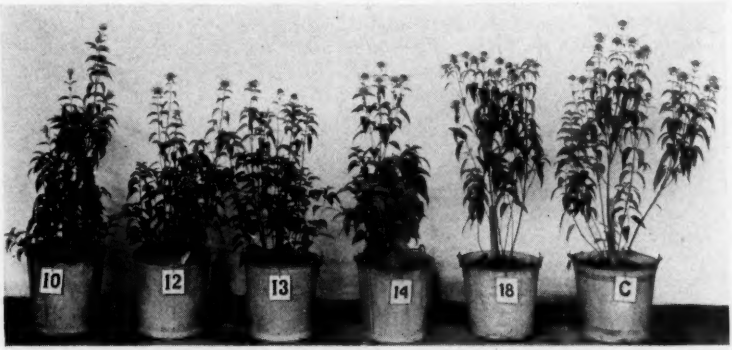

FIGURE 2.—*Monarda punctata* L. exposed to various day lengths. 10-hour day: Budded June 29; flowered June 23 at 33 inches. 12-hour day: Budded June 15; flowered July 10 at 26 inches. 13-hour day: Budded June 11; flowered June 28 at 22½ inches. 14-hour day: Budded June 15; flowered July 23 at 28 inches. 18-hour day: Budded June 14; flowered July 2 at 29 inches. Full day (C): Budded June 1; flowered July 5 at 33 inches. Tests began May 2. Photographed July 25.

lengths, but this retardation was accompanied by a decided increase in the formation of stolons, both as to number and length. This type of stem represents a prostrate form of stem growth, tending toward a more purely vegetative expression, since these stems are characterized

FIGURE 3.—*Mentha arvensis* L. exposed to various day lengths. 10-hour day: Budded July 20; flowered July 29 at 24 inches. 12-hour day: Budded August 2; flowered August 7 at 28 inches. 13-hour day: Budded July 20; flowered July 29 at 28 inches. 14-hour day: Budded July 22; flowered July 29 at 32 inches. 18-hour day: Budded August 7; flowered August 23 at 42 inches. Full day (C): Budded August 7; flowered August 27 at 36 inches. Tests began May 2. Photographed July 25.

by lengthened internodes, greatly reduced leaves, and a flowerless condition. The last-named tendency would indicate a marked reduction in sexual reproductive energy, and to this degree the short days

were unfavorable to the development of large vigorous plants and the formation of flowers.

Mentha arvensis gives more indication of being an indeterminate or day-neutral type of plant than any other species of this genus shown

FIGURE 4.—*Mentha piperita* var. exposed to various day lengths. Originally obtained from Greensboro, N. C., probably a variety of *M. piperita*. 10-hour day: Budded July 29; flowered August 5 at 23 inches. 12-hour day: Budded July 20; flowered July 30 at 27 inches. 13-hour day: Budded July 13; flowered July 23 at 30 inches. 14-hour day: Budded July 13; flowered July 29 at 30 inches. 18-hour day: Budded July 10; flowered July 23 at 32 inches. Full day (C): Budded July 9; flowered July 23 at 28 inches. Tests began May 2. Photographed July 25.

in table 1. This strain of *M. arvensis* has given some indication of delay in flowering in response to the longer light periods, and there has been a rather consistent increase in height of stem. The number of stolons produced has shown no particular relation to length of day.

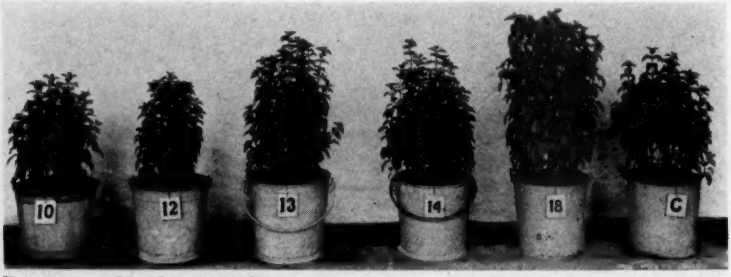

FIGURE 5.—*Mentha citrata* Ehrh. exposed to various day lengths. 10-hour day: Never budded. 12-hour day: Never budded. 13-hour day: Budded August 27; flowered September 23 at 28½ inches. 14-hour day: Budded August 23; flowered September 23 at 31 inches. 18-hour day: Budded July 29; flowered September 3 at 34 inches. Full day (C): Budded August 14; flowered September 16 at 31 inches. Tests began May 1. Photographed July 25.

Mentha citrata (fig. 5) is typically a long-day type of plant, failing to bud or flower on daylight periods of 10 and 12 hours and flowering after great delay in response to 13 hours, 14 hours, and the full length of day. The longest light period of 18 hours hastened flowering in advance of all others, an amount equal to 20 days over those plants experiencing 13 and 14 hours of light daily.

On the shorter lengths of day, more especially those of 10 and 12 hours, where flowering was inhibited, the erect flowerless stems were very leafy, and stolon development was very vigorous, indicating that the energies of growth were almost purely vegetative.

With 18 hours of light daily, vegetative expression was reduced, and sexual reproduction or flowering became the dominant expression, accompanied by weak stolon development, which is always a purely vegetative phase.

Mentha piperita (fig. 6), the true peppermint of commerce, is more pronounced in its long-day tendencies than *M. citrata*, since no buds

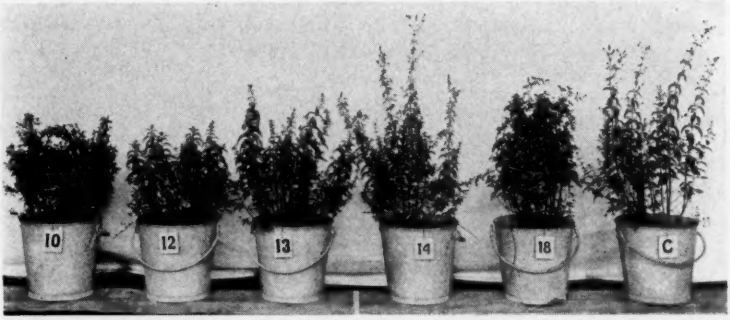

FIGURE 6.—*Mentha piperita* L. (peppermint) exposed to various day lengths. 10-hour day: Never budded. 12-hour day: Never budded. 13-hour day: Never budded. 14-hour day: Budded July 13; flowered July 29 at 31 inches. 18-hour day: Budded July 12; flowered July 29 at 27 inches. Full day (C): Budded July 9; flowered July 29 at 31 inches. Tests began May 2. Photographed August 21.

were produced until a length of day of 14 hours had been experienced. Flowering was entirely inhibited in the tests involving 10, 12, and 13 hours of light daily, no buds being formed in response to these light periods. With 14 hours of light, 18 hours, and full day, buds and flowering occurred at about the same time. Even with 14 hours of light daily, flowers were barely able to develop, one stem alone finally producing a few flowers and all other stems remaining purely vegetative.

There was a very noticeable difference in the behavior of the plants experiencing full day as compared with those experiencing 18 hours of light each day. The former had entirely ceased flowering by September 16, whereas the latter were in full flower and very floriferous.

Stolon formation was especially vigorous on the 10- and 12-hour tests, where flower development was inhibited, but had begun to decrease with 13 hours of light. With 18 hours of light and with full day, stolon formation had reached its lowest values, both in number and in length.

Mentha piperita appears to be a species most favored by the long days of high latitudes with respect to flowering and the production of erect leafy stems. In the Washington region flowering was rather brief on the full length of day.

Mentha spicata (*M. viridis* L.) (fig. 7), producing spearmint oil, flowered under all lengths of day except that of 10 hours, when no buds

were produced. There appeared to be some delay in flowering with 12 hours of light, a response perhaps to be expected since the short day of 10 hours had yielded a purely vegetative expression. Stolons were very numerous and long on the shorter lengths of day and were much fewer and shorter as the longer daylight periods were experienced. Spearmint gives stronger indications of an adaptation to the shorter days of lower latitudes than peppermint (*M. piperita*) has shown.

The plant designated *Mentha piperita* var. flowered under all lengths of day in the tests but with perhaps a slight delay with 10 hours of light daily. On this short light period there is evidence that vegetative expression was being favored, since stolon development was heaviest and

FIGURE 7.—*Mentha spicata* L. (*M. viridis* L.) (spearmint) exposed to various day lengths. 10-hour day: Never budded. 12-hour day: Budded July 1; flowered July 29 at 25½ inches. 13-hour day: Budded June 21; flowered July 12 at 25 inches. 14-hour day: Budded June 19; flowered July 7 at 26 inches. 18-hour day: Budded June 18; flowered July 8 at 29 inches. Full day (C): Budded June 15; flowered July 10 at 27 inches. Tests began May 1. Photographed August 21.

the longest runners were produced. With each increase in length of day above 10 hours, stolon development declined until with 18 hours of light only two short runners developed.

If this mint represents a variety of *Mentha piperita*, it would appear that the wide range of flowering, extending far down into the shortest light periods, is more nearly like the behavior of *M. arvensis* than that of *M. piperita* (table 1) and indicates the marked differences that closely related varieties of a species may show in their reaction to the factor of length of day.

CONCLUSIONS

The present paper shows the day-length requirements of a number of strains of species of *Monarda* and *Mentha* grown at the Arlington Experiment Farm, Arlington, Va. Some of these are of Old World origin, and a number, including *Mentha spicata* and *M. piperita*, are thoroughly naturalized in the East.

Monarda punctata, *Mentha spicata*, and *M. piperita* occur wild in the District of Columbia; *Monarda didyma* occurs wild in the adjacent area of Virginia.

Some of these mints have very definite length-of-day requirements for flowering, and the various species differ widely in this respect. Some are obviously best adapted to northern regions with long days,

while others can flower readily in lower latitudes where much shorter summer days prevail.

The species *Monarda punctata* and *M. didyma* have flowered readily under all light periods and behave as indeterminate or day-neutral plants, their flowering being little affected by the length of the daily light periods. On the basis of their readiness to flower in response to various day lengths, ranging from 10 to 18 hours, it should be expected that both species would have a wide natural range of distribution southward and northward. That such is the case is shown by the fact that *M. didyma* is found from Quebec, Canada, to Florida, and *M. punctata* from New York to Florida and Texas. It is probable that at the limits of their range, in both the extreme north and the extreme south, the temperature factors may play an important part in their delimitation.

The several species of *Mentha*, for the most part, are typical long-day plants in their day-length responses, since some flowered more quickly when the longer lengths of day were experienced and others flowered only under these longer day lengths. Of the five strains or species tested, *M. piperita* showed the most pronounced requirement for long days, since flowering was not initiated even with 13 hours of light daily and flowers were barely able to form when the plants were given 14 hours of light daily. In the case of *M. citrata*, the lower limits of flowering were shown with 12 hours of light daily, and plants of *M. spicata* failed to flower with 10 hours of light daily.

It is important to recognize the fact that the plants of the various species tested probably represent particular strains in all instances and that these results would not necessarily apply to all other strains of these species found in this country or in the Old World.

As has been indicated, most of the strains of the *Mentha* species dealt with in this paper have shown more or less pronounced long-day requirements so far as free flowering is concerned. The recognition of this fact is of much importance to the plant breeder who may hope to hasten the flowering of certain species or to synchronize their flowering to best advantage for purposes of ready crossing by modifying the daily light period. It is obvious that shortening the days to hasten flowering in some of these species, as one plant breeder interested in mint breeding informed the writer he had done, is anything but the correct procedure.

The writer's studies of these mints have been concerned only with the ecology of growth and flowering, but the percentage yields of aromatic oil and its composition in relation to the various lengths of day would also have been of considerable interest.

It is evident that the various species have shown strongly contrasting responses to the various lengths of day, and it is true generally that where flowering was inhibited on the shorter days stolon development and elongation were favored.

The species of *Mentha* for the most part produce two types of stems, one of erect habit bearing the normal leafage and capable of producing flowers under suitable conditions; the other a prostrate, stoloniferous, nonflowering type, with long internodes and small leaves, which may grow to excessive length and root readily along its course when in contact with the ground. It is obvious that the latter is a strictly vegetative, propagative type of stem, which serves to extend the colonies into new areas.

Such evidence as has been secured would indicate that, with suppression of the flowering tendency under shortened day lengths, the stolon type of stem development is favored. An undue accentuation of this type of stem, with restriction of erect stem growth, obviously would tend to lower the production of plant material per acre and consequently the yield of aromatic oil. It would appear, then, that maximum yields, percentage composition being equal, would obtain in those areas most favorable to maximum erect stem growth, which to a greater or less degree is related to favorable length of day. For optimum conditions of flowering and for early flowering long days are essential. This is probably one of the principal reasons why peppermint (*Mentha piperita*) succeeds best in northern latitudes.

SUMMARY

The length-of-day requirements of a number of mints of the genera *Monarda* and *Mentha* of the family Labiatae were studied, the range of photoperiods being 10, 12, 12½, 13, 13½, 14, 14½, 18 hours, and full day, at Arlington, Va., at approximately lat. 39° N.

Natural daylight was used for all photoperiods except that of 18 hours, this being much in excess of the longest natural day at Washington, D. C., which on June 21 is 14.9 hours.

For photoperiods of 10 to 14½ hours, inclusive, the plants were kept in ventilated lightproof houses for definite periods each day to obtain the desired number of hours of daylight exposure.

For the 18-hour photoperiod, artificial supplemental light was afforded the plants by four 200-watt gas-filled tungsten lights fitted with bowl reflectors, one light being placed at each corner of a square metal frame 3 feet on a side from center to center of the lights. These lights were kept at a distance of 1 foot from the plants by raising the supporting metal frame as needed. The light intensity thus afforded was about 300 to 400 foot-candles as measured by a Weston illumination meter, model 1746, equipped with a Viscor filter measuring visible radiation only.

The species studied were *Monarda didyma*, *M. punctata*, *Mentha arvensis*, *M. citrata*, *M. piperita*, *M. spicata*, and a variety of *M. piperita*.

Most of these mints require long days for early profuse flowering, but the various *Mentha* species, which are characterized by the formation of long stolons under some conditions, show accentuation of the stoloniferous habit in some instances on the shorter days.

The two *Monarda* species, *M. didyma* and *M. punctata*, are day-neutral or indeterminate in their behavior, since the most extreme differences in the length of the photoperiod had very little effect on the height and time of flowering.

Mentha citrata, *M. piperita*, and *M. spicata* have shown the most decided long-day tendencies, since flowering was inhibited entirely on shortened days. Of these, *M. piperita* was most favored by long days, since only the photoperiods of 14 hours, 18 hours, and the full day allowed buds and flowers to appear.

Mentha arvensis and a variety of *M. piperita* obtained from Greensboro, N. C., produced flowers even in response to the shortest photoperiod of 10 hours, and to this extent both are inclined to be indeterminate or day-neutral in their length-of-day responses.

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